ISSN-0973-9122 (Print) ● ISSN-0973-9130 (Electronic)

Volume 14 / Number 2 / April-June 2020

Indian Journal of Forensic Medicine & Toxicology

Website: www.ijfmt.com

Covered by Scopus ★ EMBASE ★ Approved by MCI

Official Organ of Indian Association of Medico-Legal Experts (Regd.)

Indian Journal of Forensic Medicine & Toxicology

Print-ISSN:0973-9122 Electronic - ISSn: 0973-9130

Website: www.ijfmt.com

Editor

Dr. R.K. Sharma
Institute of Medico-legal Publications
Logix Office Tower, Unit No. 1704, Logix City Centre Mall, Sector- 32, Noida - 201 301 (Uttar Pradesh)

Printed, published and owned by

Dr. R.K. Sharma
Institute of Medico-legal Publications
Logix Office Tower, Unit No. 1704, Logix City Centre Mall, Sector- 32, Noida - 201 301 (Uttar Pradesh)

Published at

Institute of Medico-legal Publications
Logix Office Tower, Unit No. 1704, Logix City Centre Mall, Sector- 32, Noida - 201 301 (Uttar Pradesh)

"Indian Journal of Forensic Medicine & Toxicology" is peer reviewed quarterly journal. It deals with Forensic Medicine, Forensic Science, Toxicology, DNA fingerprinting, sexual medicine and environment medicine. It has been assigned International standard serial No. p-0973-9122 and e- 0973-9130. The Journal has been assigned RNI No. DELENG/2008/21789. The journal is indexed with Index Copernicus (Poland) and is covered by EMBASE (Excerpta Medica Database). The journal is also abstracted in Chemical Abstracts (CAS) database (USA. The journal is also covered by EBSCO (USA) database. The Journal is now part of UGC, DST and CSIR Consortia. It is now offical publication of Indian Association of Medico-Legal Experts (Regd.).

Indian Journal of Forensic Medicine & Toxicology

Contents

Volume 14, Number 2 **April-June 2020**

Morphology of Palatal Rugae in Various Sagittal Skeletal Malocclusions in Kerala Population- A Retrospective Study

Crystal Runa Soans[1], Azhar Mohammed[2], Murali PS[1], Mcqueen Mendonca[3], Prajwal Shetty[3], VartikaKumari[4]

[1]Senior lecturer, [3]Reader, [4]Ex-Post Graduate, Department of Orthodontics and Dentofacial Orthopaedics, A.B Shetty Memorial Institute of Dental Sciences, NITTE (deemed to be) University, Mangalore, India.

Abstract

The present study was designed evaluate the structural morphology of palatal rugae in Kerala orthodontic subjects with varying Sagittal Skeletal Malocclusions. Pretreatment maxillary casts of 105 patients were analyzed for rugae patterns qualitatively and quantitatively using modified Thomas and Kotze classification. The data were statistically analyzed. According to the Fishers exact test, the most common type of rugae pattern in all the groups (Class I, Class II, and Class III) were observed to be wavy followed by curved. Skeletal Class III had the highest number of straight rugae 71.4% among the three groups which was statistically significant. Direction of rugal alignment was horizontal and droplet-shaped incisive papilla was identified among different skeletal dysplasia groups.Palatal ruage may serve as valuable indicators for population identification and help in facial reconstructions through their association with varying skeletal malocclusions.

Keywords: *Palatal Rugae Pattern, Skeletal Malocclusion, Dental Casts, Forensic Dentistry*

Introduction

The irregular asymmetrical palatine folds on the anterior third of the palate are called as palatal rugae. They possess unique individualistic morphologic characteristics that are helpful in population identification and are stable over time.[1, 2]

In a county like India where usable dental anthropological data is sparse palatal rugae could be valuable for forensic identification due to racial specificity, facilitating population identification and post- mortem identification.

Rugae are classified based on their length, shape, direction of alignment, unification or branching number distributed on either side of the median palatal raphae. The plica palatinae are thus assessed qualitatively and quantitatively based on these parameters.

Studies have shown the uniqueness of the rugae when assessed in various populations and may be helpful in individual identification and population identification.[3- 7]

Stability of palatal rugae, pre and post orthodontic treatment have made them reliable markers of identification. Studies have also attempted to establish the relation of palatal rugae pattern and various dental malocclusions, thereby allowing for early intervention and prevention of dento-skeletal issues.[8, 9]Astudy was conducted to investigate morphological structure of palatal rugae in Turkish orthodontic subjects with various sagittal skeletal malocclusions, concluded the uniqueness of rugae morphology in relation to skeletal dysplasia.[10]

No study has been conducted on individuals with various sagittal skeletal malocclusions in Indian population with varying ethnicities. The purpose of this study was to evaluate the morphological structure

Corresponding Author:
Azhar Mohammed,
Senior Lecturer, Department of Orthodontics and Dentofacial Orthopaedics, A.B Shetty Memorial Institute of Dental Sciences, NITTE (deemed to be) University, Mangalore, India.
E-mail: dr.azhar.mohd@gmail.com

of palatal rugae in Kerala orthodontic subjects with different sagittal skeletal malocclusions.

The objective of the present study was to evaluate and compare the structural morphology (number, pattern, direction of alignment) of palatal rugae and shape of the incisive papilla in Kerala orthodontic subjects with varying sagittal skeletal malocclusions i.e. Skeletal Class I, Skeletal Class II and Skeletal Class III.

Material and Method

The study was designed to be a retrospective study. Pretreatment records i.e lateral cephalograms and maxillary casts of 105 subjects age ranging 18-25 years, were procured from the archives of the department. Records of patients with any congenital deformity or history of palatal surgery and previous history of orthodontic treatment were excluded. The maxillary models whose palatal rugae were clearly visible were selected.

Methodology

The subjects were grouped into Class I, Class II, Class III (n=35 each) according to the ANB angle measured on lateral cephalometric radiographs. (Class I ANB angle 0º to 4º, Class II ANB angle >4º and Class III ANB angle <0º).

The maxillary casts were numbered. A midline was drawn coinciding with that of the mid palatine raphae extending from the incisive papillae to the posterior most extent of the rugae dividing the palate into right and left halves. The outlines of the rugae were traced using graphite pencil under adequate illumination. The rugae were then measured and categorized as primary, secondary and tertiary rugae and assessed for different patterns. Modified Thomas and Kotze[11] method was used for quantitative and quantitative assessment. The number, pattern, direction of rugae alignment and shape of the incisive papilla were assessed among all the groups.

Statistical Analysis

All data were analysed using IBM SPSS software VERSION 22.0. The Kruskal–Wallis test was used to compare the number of primary palatal rugae (PPR) among the groups.Fishers exact test was used to compare the pattern of rugae between the various skeletal malocclusions on both sides of the palate and the direction of rugae among the groups. Chi square test was used to compare the shape of the incisive papilla among the groups. P values of less than 0.05 were considered statistically significant.

Results

Kruskal- Wallis test analyzed the primary palatal rugae (PPR) quantitatively and compared among the groups on the right and left side of the palate. As regards to the number of PPR on the left and right sides, there was maximum number of primary palatal rugae on the right side of subjects with and Skeletal Class II (mean 3.86right and 3.80 left) and Skeletal Class III (mean 4.17 right and 3.97 left) in comparison with Skeletal I(mean 3.54 right and 3.60 left) with the highest number of primary rugae in Skeletal Class III on the right side among the groups, which was statistically significant (p value 0.003) (Table 1).

Table 1: Kruskal Wallis test showing the number of primary palatal rugae (PPR) among the groups

Primary	Class	N	Mean (SD)	Range	Median (Q1-Q3)	Chi square value	p-value
Right	**Class 1**	35	3.54 (0.61)	2 - 5	4(3 - 4)	11.90	0.003*
	Class 2	35	3.86 (0.65)	3 - 5	4(3 - 4)		
	Class 3	35	4.17 (0.79)	3 - 6	4(4 - 5)		
Left	**Class 1**	35	3.60 (0.55)	3 - 5	4(3 - 4)	4.33	0.12(NS)
	Class 2	35	3.80 (0.58)	3 - 5	4(3 - 4)		
	Class 3	35	3.97 (0.89)	2 - 6	4(3 - 5)		

N=Samples In Each Group*p<0.05 statistically significant,

Wavy and curved types were the most common types of rugae pattern in all skeletal dysplasia groups. On the right side (Class I,93%wavy and 31.4%curved, Class II,91%wavy and 51.4% curved, Class III,80%wavy and 65.7%curved).Followed by straight and unification pattern. Skeletal Class III had the highest number of straight rugae 71.4% among the three groups which was statistically significant (p value 0.00) (Figure 1). On the left side of the palate Wavy pattern was the most common pattern in all malocclusion groups (Class I, 94.3%wavy, Class II 94.3%wavy, Class III, 77.1%), but this was not statistically significant.Followed by curved, straight and unification pattern. Skeletal Class III had the highest number of straight rugae 62.9%among the three groups which was statistically significant (p value 0.003). (Figure 2)

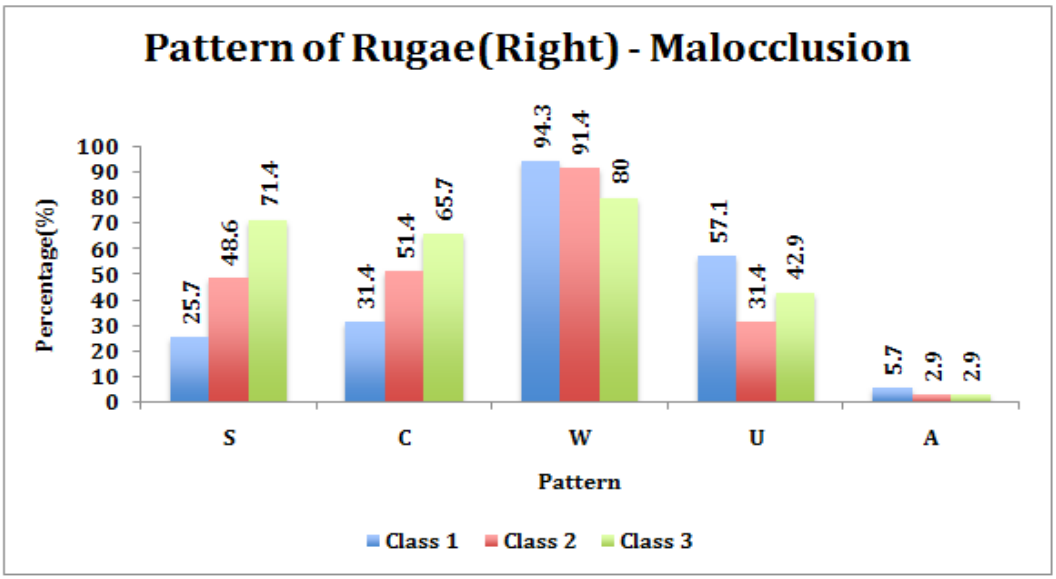


Figure 1: Graph showing the pattern of rugae on right side among the groups.

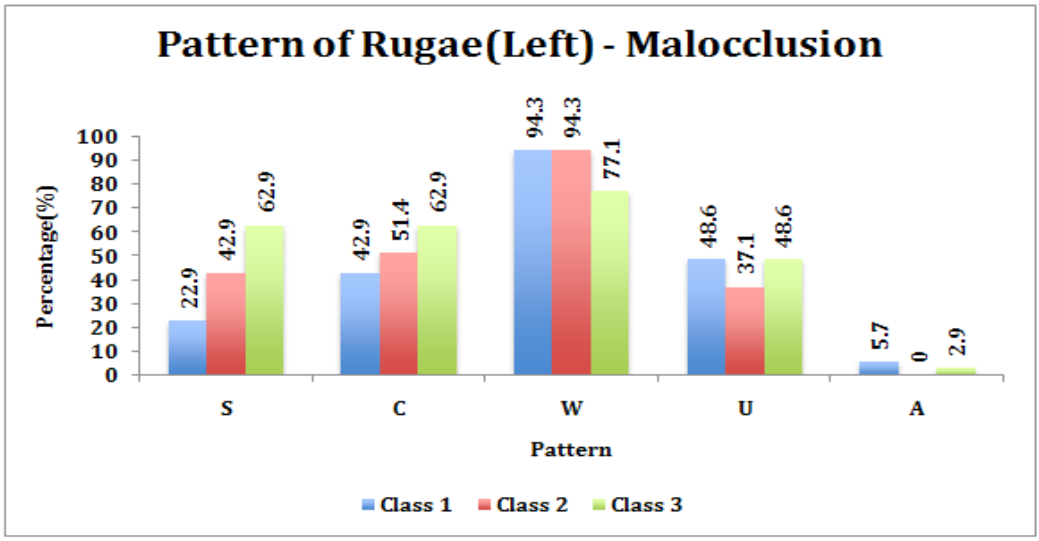


Figure 2: Graph showing the pattern of rugae on left side between the groups

Fischer's exact test compared the direction of rugae among the groups. Direction in all groups was horizontal (54.3% Class I, 85.7% Class II, 68.6% Class III).Irregularly directed rugae were seen highest in Skeletal Class I group(40.0%) as compared to the other groups and this was statistically significant (p value 0.003). (Figure 3).

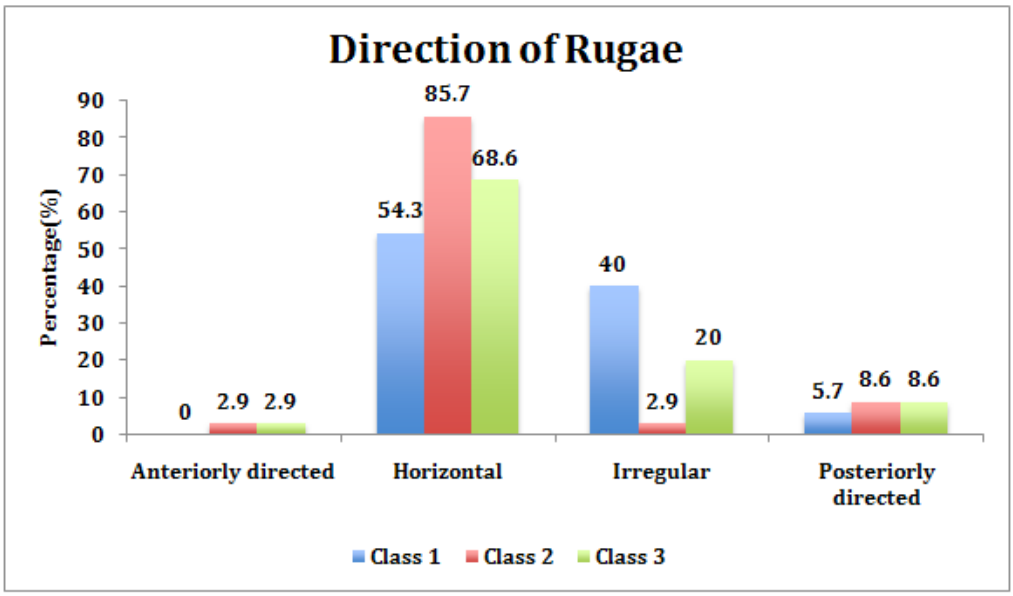


Figure 3: Fisher's Exact Test showing the direction of rugae alignment in different skeletal malocclusions among the groups

Droplet-shaped incisive papilla was identified to be predominant among different skeletal malocclusion groups. (Table 2).

Table 2:-

Shape of incisal papilla	Malocclusion			Total
	Class 1	Class 2	Class 3	
Cylindrical	11	7	8	26
	31.4%	20.0%	22.9%	24.8%
Droplet	24	28	27	79
	68.6%	80.0%	77.1%	75.2%
Total	35	35	35	105
	100.0%	100.0%	100.0%	100.0%
Chi square test	Chi square value = 1.33(2), p-value =0.52(NS)			

*p<0.05 statistically significant, p>0.05 Non significant, NS

Table 2: Chi Square Test showing the shape of the incisive papilla among the groups

Discussion

Palatal rugae with their individual uniqueness have aided in forensic identification and population identification and have imposed their importance in cases were digit biometrics are unavailable. The shape of the palatal rugae seems to remain constant throughout life.[12]Palatal rugae facilitate population identification as they possess identical characteristics amongst certain racial groups.[13]Like fingerprints the palatal rugae do not alter during the course of life with only increase in length due to normal growth.[14]

Even though examination of the palatal rugae is relatively a subjective process, it can be easily recorded sans any complex armamentarium. Palatal rugae are unique in each individual similarly, in this present study, each subject's palatal rugae were found to be unique.[15-18]

Some authors suggest that palatal rugae remain unchanged as of 12th weeks of intrauterine life; whereas other authors suggest that palatal rugae may change quantitatively with increasing age but retain their general configuration.[19, 20]

In the present study we only assessed the number and shape of the palatal rugae among different skeletal malocclusion groups and no time related evaluation of the rugae was undertaken.

The number of PPR ranged from 2-6 and SPR ranged from 0-5 in the subjects examined in the present study. Study done by Surekha et al[5] showed more number of PPR in Kerala population as compared with Manipuri population. In addition, there was significant difference among different Sagittal Skeletal Malocclusion groups in terms of the overall number of PPR on either sides of the palate. Primary rugae were distributed more on the right side of the palate among the groups, especially in Skeletal Class II and Skeletal Class III. These results concur with those of Kallianpur et al [21]who compared rugae patterns of Indian and Nepalese population. Rugae were higher in number on the right side of the palate in Indians as compared to Nepalese population but the results were not statistically significant. Dhoke and Osato[22] and Kapali et al [13], explained the phenomenon of regressive evolution that dominates the right side of the palate mainly pertaining to secondary rugae. Our results may thus vary due to the exclusion of secondary rugae and based on the population chosen.

According to Thomas 1983, palatal rugae do not undergo any changes except in length, throughout a person's life probably due to underlying growth.[11]Comparative studies performed amongst geographically diverse populations belonging to Indian regions such as Karnataka and Kerala[23] and Manipur and Kerala[5], have demonstrated important differences in rugae pattern. A study comparing two groups of Indian population (southern Indians and western Indians) predominantly showed the prevalence of wavy and curved rugae forms followed by straight rugae pattern.[24]Studies conducted by Nayak et al[25],Kotrashetti el al[26], Kumar el al[27],Surekha et al[5],Shanmugam et al[7], Bajracharya et al[28] and Kapali et al[13]showed the predominance of curved and wavy pattern of rugae in most populations. The Present study was aimed more towards assessing the characteristics of rugae in various skeletal malocclusions in Kerala population. Wavy pattern was seen most predominantly on both sides in Class I followed by Class II and Class III skeletal malocclusion. These are in accordance with the results published by Surekha et al[5] and M Selvamani et al[29] and SwethaS[30]. This was followed by curved, straight and unification pattern of rugae. Study by J K Savitha et al[23] has seen similar results where curved rugae were more prominent in Kerala population as compared to Karnataka population. Horizontal rugae alignment and droplet-shaped incisive papilla was identified among all three skeletal dysplasia groups.The study by Ekrem Oral et al[10] also reported droplet-shaped incisive papilla to be the most common type in Turkish population. M Selvamani et al [29]concluded that females of Kerala origin had more number of primary rugae as compared to males. In our study we have not compared for sexual dimorphism among the various skeletal malocclusion. This leaves room for future scope of the study.

Further studies on a large number of subjects of varying Indian ethnicities are required in order to confirm the relationship between the pattern of palatal rugae and sagittal skeletal malocclusion.

Conclusions

- Primary rugae were significantly distributed more on the right side in comparison to the left side on the palate, especially in skeletal Class II and Class III.
- Wavy and curved pattern of the rugae were seen on both right and left side of all the skeletal malocclusion groups and skeletal Class III had more number of straight rugae.
- Direction of rugae alignment was horizontal and droplet-shaped incisive papilla was identified among different skeletal dysplasia groups.
- Palatal ruage may serve as valuable indicators for population identification and help in facial reconstructions through their association with varying skeletal malocclusions.

Ethical Clearance: Ethical clearance was obtained from the 'Ethics Committee' of the Institution prior to the start of the study.

Source of Funding: Self

Conflict of Interest: Nil

References

1. Thomas CJ, Kotze TJ, Van der Merwe CA. An improved statistical method for the racial classification of man by means of palatal rugae. Arch Oral Biol. 1987;32:315-7.
2. Allen H. The palatal rugae in man. Dental Cosmos

1889;31:66-80.

3. Saraf A, Bedia S, Indurkar A, Degwekar S, Bhowate R. Rugae patterns as an adjunct to sex differentiation in forensic identification. J Forensic Odontostomatol. 2011;29:14–9.
4. Bharath ST, Kumar GR, Dhanapal R, Saraswathi T. Sex determination by discriminant function analysis of palatal rugae from a population of coastal Andhra. J Forensic Dent Sci. 2011;3:58–62.
5. Surekha R, Anila K, Reddy VS, Hunasgi S, Ravikumar S, Ramesh N. Assessment of palatal rugae patterns in Manipuri and Kerala population. J Forensic Dent Sci. 2012;4:93–6.
6. Limson KS, Julian R. Computerized recording of the palatal rugae pattern and an evaluation of its application in forensic identification. J Forensic Odontostomatol. 2004;22:1–4.
7. Shanmugam S, Anuthama K, Shaikh H, Murali K, Suresan V, Nisharudeen K, et al. Palatal rugae in population differentiation between South and North Indians: A discriminant function analysis. J Forensic Dent Sci. 2012;4:75–9.
8. Shukla D, ChowdhryA, Bablani D, Jain P, Thapar R. Establishing the reliability of palatal rugae pattern in individual identification (following Orthodontic treatment).J Forensic Odontostomatol.2011;29:1:20-29.
9. Kapoor P, Ragini, Kaur H. Rugoscopy: A Diagnostic Appurtenance for Malocclusion or just a Forensic Aid? - A Pilot Study. J Forensic Res. 2015; 6:272.
10. Oral E, Buyuk SK, Simsek H. Evaluation of palatal rugae pattern in different sagittal skeletal relationship adolescent subjects. Medicine (Baltimore).2017;96(14)
11. Thomas C, Kotze T. The palatal ruga pattern in six southern African human populations. Part I: a description of the populations and a method for its investigation. J Dent Assoc S Afr. 1983;38:158–65.
12. Abou El-Fotouh MM, El-Sharkawy GZ.A study of palatal rugae pattern (rugoscopy) in an Egyptian population. J Egypt Dent Assoc. 1998;44:3177–84.
13. Kapali S, Townsend G, Richards L, Parish T. Palatal rugae patterns in Australian aborigines and Caucasians. Aust Dent J. 1997 Apr;42(2):129-33.
14. Almeida MA, Phillips C, Kula K, Tulloch C.Stability of the palatal rugae as landmarksfor analysis of dental casts. Angle Orthod.1995;65: 43-8.
15. Eboh DE. Palatal rugae patterns of Urhobos in Abraka, South-Southern Nigeria.Int J Morphol. 2012;30:709–13.
16. Saraf A, Bedia S, Indurkar A, et al. Rugae patterns as an adjunct to sex differentiation in forensic identification. J Forensic Odontostomatol. 2011;29:14–9.
17. Gandikota C, Venkata YP, Challa P, et al. Comparative study of palatal rugae pattern in class II div 1 and class I individuals. J Pharm BioalliedSci.2012;4:S358–363.
18. Rath R, Reginald BA. Palatal rugae: an effective marker in population differentiation. J Forensic Dent Sci. 2014;6:46–50.
19. Lysell L. Plicaepalatinaetransversae and papilla incisiva in man; a morphologic and genetic study. ActaOdontScand. 1955;13:5–137.
20. Simmons J, Moore R, Erickson L. A longitudinal study of anteroposterior growth changes in the palatine rugae. J Dent Res. 1987;66: 1512–5.
21. Kallianpur S, Desai A, Kashetty S, Sudheendra U, Joshi P. An anthropometric analysis of facial height,archnlength, and palatal ruage in the Indian and Nepalese population. J Forensic Dent Sci..2011; 3(1): 33-37
22. Dhoke M, Osato S. Morphological study of the palatal rugae in Japanese.Bilateral difference in the regressive evolution of the palatal rugae.Jpn J Oral Biol. 1994;36:126–40.
23. Savita JK, Yathindra Kumar BN, Satish G, Divya KT, Ranjitha J, PujariRK.Prevalence of palatal rugae shapes in Karnataka and Kerala population: A cross-sectional study. J IntSocPrev Community Dent. 2016;6(3):230
24. Nayak P, Acharya AB, Padmini AT, Kaveri H. Differences in the palatal rugae shape in two populations of India. Arch Oral Biol. 2007;52(10):977-82.
25. Kotrashetti VS, Hollikatti K, Mallapur MD, Hallikeremath SR, Kale AD. Determination of palatal rugae patterns among two ethnic populations of India by logistic regression analysis. J Forensic Leg Med. 2011;18(8):360-5.
26. Kumar S, Vezhavendhan N, Shanthi V, Balaji N, Sumathi MK, Vendhan P. Palatal Rugoscopy

among Puducherry Population. J Contemp Dent Pract. 2012;13(3):401-404.

27. Bajracharya D, Vaidya A, Thapa S, ShresthaS. Palatal Rugae Pattern in Nepalese Subjects. Orthodontic Journal ofNepal 2014;3(2):36-39.
28. Kapali S, Townsend G, Richards L, Parish T. Palatal rugae patterns in Australian aborigines and Caucasians. Aust Dent J. 1997;42(2):129-33.
29. Selvamani M, Hosallimath S, Madhushankari, Basandi PS, Yamunadevi A. Dimensional and morphological analysis of various rugae patterns in Kerala (South India) sample population: A cross-sectional study. Journal of Natural Science, Biology, and Medicine. 2015;6(2):306-309.
30. Swetha, S. Palatal Rugae pattern in varied adult Indian population of Males and Females. Journal of Pharmaceutical Sciences and Research. 2015:7. 736-739.

Analysis of Hospital Deaths at Tertiary Care Teaching Hospital

Jeeveswararao Bagadi[1] , Srinivasulu Pothireddy[2] Sujan Kumar Mohanthy[3]

[1]Assistat Professor, [2]Professor & Head, Department of Forensic Medicine & Toxicology, Great Eastern Medical College, Srikakulam, Andhra Pradesh, [3]Associate Professor, Fakhir Mohan Medical College and Hospital, Balasore, Odisha

Abstract

Recording of Death and its causes constitute an important component of health information system. The study was conducted on 90 hospital deaths during the period of six months from July-December of 2017, at Great Eastern Medical School and Hospital. Males more than 60 years of age group were the major victims in our study. Improving primary health care facilities in rural areas may reduce the Mortality and Morbidity.

Key words:- *Sudden Death, Mortality, Morbidity.*

Introduction

Thanatology is a branch of science that deals with the study of death[1]. A good death is not a single event; it is a series of events. Death analysis gives the circumstances and cause of death of patient and steps to be taken for prevention of same [2].

India is undergoing rapid transition as a consequence of economical and social reforms[3]. Life expectancy at birth in India shows a continuous increasing trend from 23.63 years for male and 23.96 years for female in 1901 to 66.9 years for males and 70.0 years for females in 2011[4]. The pattern of diseases in developing countries is different than developed ones. In India about 40% of deaths are from infectious, parasitic and respiratory diseases as compared with 8% in developed countries[5].

Death analysis determines the causes of major illness, quality of medical care provided to patients from analyzing the clinical records and hospital services[2]. The pattern of mortality is a key indicator of the consequent health scenario[3].

Hospital based death records provide information regarding the causes of deaths, case fatality rates, age and sex distribution, which are of great importance in planning health care services[5] . Mortality pattern is poorly documented in rural areas lacking retention of up-to-date medical records[6]. In 19th and early 20th century communicable diseases dominated the health problems, in recent years non communicable diseases account for half of all deaths in developing countries. The main 4 killers of non communicable diseases are Cardio vascular diseases, Cancer, Diabetes and Chronic Lung Diseases [6].

Material and Method

A retrospective study was conducted at Great Eastern Medical School and hospital Srikakulam, a referral, tertiary care teaching hospital. All deaths that occurred during 6 months period from July to December of the year 2017 were considered for this study. Total 9,436 cases were admitted and 90 deaths were recorded. Age, Gender, Place of residence, Date of admission, Date of death, Survival period, Socio economic status, Family history, Past history, Chronic diseases, Proper basic patient care like CPR,ECG, were done or not, Consent taken or not, MCCD form properly filled or not and cause of death were recorded in this study. Approval of institution ethics committee was obtained prior to the study.

Table: 1 Social factors of hospital deaths

Age >60	37.77%

Corresponding author:-
Srinivasulu Pothireddy
Professor and Head, Department of Forensic Medicine ad toxicology, Great Eastern Medical School, Srikakulam, A.P. Email: forensic1pokiri@gmail.com

Male	61.11%
Hindu	94.44%
Rural	88.88%
Low socio economic	84.44%
Married	68.88%
Uneducated	72.22%

Table: 2 Age of patients in hospital deaths

S.No	Age	No of Deaths	%
1	0-10	12	13.33%
2	11-20	3	3.33%
3	21-30	7	7.77%
4	31-40	6	6.66%
5	41-50	13	14.44%
6	51-60	15	16.66%
7	>60	34	37.77%

Table: 3 Time of Deaths

S.No	Time	No of Deaths	%
1	8AM -12PM	26	28.88
2	1PM – 8PM	36	40.00
3	9PM - 12AM	9	10.00
4	1AM – 7AM	19	21.11

Table: 4 Period of survival of patients in hospital deaths

S.No	Time Period	No of Deaths	%
1	<24hrs	34	37.77
2	1-3 days	27	30.00
3	>3-<5 days	8	8.88
4	>5-<10 days	7	7.77
5	>10-<15 days	6	6.66
6	>15-<20 days	2	2.22
7	>20 days	6	6.66

Table 5: Type of system involved in Hospital deaths

S.No	System	No of Deaths
1	Respiratory	26
2	Cardiovascular	21
3	Central nervous	14
4	Gastro intestinal	8
5	Renal	7
6	Hematological	3
7	Endocrine	2
8	Multiple systems	9
9	Total	90

Table 6: List of major causes of death in hospital deaths

S. no	Cause of death	No of deaths
1	Myocardial Infarction	8
2	Acute Respiratory Distress Syndrome	8
3	Pneumonia	7
4	Sepsis	7
5	Encephalopathy	6
6	Renal Failure	6

Observations & Discussion

Deaths were more common in the age group > 60 years (37.77%) followed by 41-60 years (31.11%). This study correlates with M M kauser et al[5,] V M holamble[7.] Lowest percentage of hospital deaths were recorded in the age group 11-20 years (3.33%). More deaths after 60 yrs can be explained by the pathological basis of disease, and decreased immunity in old age to infections.

More number of male deaths were recorded (61.11%) than female deaths (38.88%). This study correlates with M M kauser et al[5.] This can be explained by more attention towards the health of male by family members. Deaths in Hindu religion (94.44%) can be explained by the more people belonging to Hindu religion in this area.

The highest numbers of Hospital deaths were in people belonging to rural area (88.88%) than urban area (11.11%). This may be because of location of the hospital is in rural area and serving more rural population. People from rural area are poor and referred from all types of medical centers like PHC, CHC, to medical college hospital in terminal stages of illness. People from urban area may have medical care from private sector. This study correlates with M M kauser et al[5] , V M holamble[7].

More deaths were recorded in uneducated people (72.22%) than in educated people. This may be due to lack of knowledge on health in uneducated people in rural areas. In people with low socioeconomic status (84.44%) highest number of deaths was recorded followed by middle class people (11.11%). This may be due to poverty, poor people may be neglecting health and don't visit the hospital for screening and awareness programs. The highest number of deaths (n=62) were in married people than unmarried (n=28). More number of married people deaths can be explained by lower marriage age in rural areas.

People admitted with one of the chronic diseases (42.22%) were suffering from Hypertension, Diabetes, Thyroid, Carcinoma, which are prone to develop complications and early death. Past history of suffering from similar disease or other chronic disease was found in (27.77%) patients. In 5.55% of patients, family history of same disease was present. The patients who have not gone through any previous treatment or any screening procedures died more (62.22%), when compared with patients taking treatment (37.77%). The patients who are under treatment may take care of their health, there by prolonging the life. A positive history of alcohol and smoking was present in 22.22% of patients.

Highest numbers of deaths were recorded during day time (68.88%) than night time (31.11%). More number of deaths were recorded in people admitted in day time (72.21%) than night time (27.77%).

Deaths with in 24 hrs of hospital admission were more (n=34) in this study, implying the importance of screening procedures in rural villages of this district to prevent sudden deaths. Death is said to be sudden or unexpected when a person not known to have been suffering from any dangerous disease, injury or poisoning is found dead or dies within 24 hours after the onset of terminal illness[8] . The incidence is approximately 10 percent of all deaths[8] ,which contrasts with our study (37.77%).

Diseases of Respiratory System killed more number of patients (n=26) followed by Cardio Vascular System (n=21), Central Nervous System (n=14), Gastro Intestinal System (n=8), Renal System (n=7), Hematology (n=3), Endocrine (n=2), and Multiple Systems (n=9). This study correlates with c.palaivel et al [9]contrasts with yogeshwar V. kalkonde et.al [10] where stroke is the leading cause of death in rural people.

Myocardial Infarction and acute respiratory distress syndrome were the major immediate causes of death. This study correlates with c.palaivel et .al[9] contrasts with yogeshwar V. kalkonde et.al [10] where stroke is the leading cause of death in rural people.

In 75.55% of cases CPR was done and flat ECG was obtained in 81.11% cases and detailed consent was taken in 65.55% cases. MCCD forms were filled in 100% of cases.

Manner of death in 96.66 % of cases was natural and in 3.33% accidental.

Cadaveric organ donation was not done in this part of state even though 28 patients died below 40 yrs of age group in this study, due to lack of awareness.

In 18 cases, in MCCD forms immediate cause of death was written as cardio respiratory arrest. It shows the doctors are not well trained in MCCD form filling.

Conclusions

1. Deaths were more common in males aged more than 60 years.

2. Most of deaths occurred in rural, uneducated, low socio economic group people.

3. Respiratory diseases followed by Cardio vascular diseases accounted for highest number of deaths.

4. Acute respiratory distress syndrome and Myocardial infarction are the major immediate cause of deaths in respiratory and cardio vascular diseases respectively.

5. Diabetes and Hypertension were the common co morbid conditions observed in this study.

6. Significant number of deaths in young (n=15) people indicates importance of preventive measures like vaccines, screening methods etc.

7. Medical services were not available /used by most of the people before reaching this hospital.

8. More than one third of deaths (n=34) were sudden deaths i.e. <24 hrs of survival period after starting of the symptoms.

9. Most of deaths were natural deaths.

Suggestions and Recommendations

1. Higher mortality among males in rural areas will retard economic growth rates. So there should be strong health policy for preventive as well as curative health services.

2. Government should initiate better health awareness campaigns for healthy life styles, environment modifications, and safety measures in rural people.

3. Airway management, fluid resuscitation along with screening procedures for respiratory and cardiovascular diseases are the early contributors to prevent sudden deaths in hospitals.

4. Continuous evaluation in hospital records provides stimulation for improvement of clinical services, professional education, hospital administration and better patient care.

5. First three days after admission is better period which needs proper medical attention to avoid preventable deaths.

6. As hypertension and diabetes are the risk factor for Myocardial Infarction, screening and treatment of patients with hypertension and diabetes in rural areas reduces Myocardial Infarction mortality.

7. Delay in diagnosis and immediate treatment, and decision to transfer to higher centre may be the factors for deaths in rural area. Government should provide proper transport and should improve health facilities in rural area.

8. Late admissions, poor maintenance of health due to poor socioeconomic status may come under contributory negligence. Government should provide health workers in rural area to screen the people and avoid the late admissions.

9. Organ donation awareness programs should be conducted to improve organ donations in rural areas.

Acknowledgement: I am thankful to Dr Srinivasulu Pothireddy (Professor and Head), and Dr Sujan Kumar Mohanthy(Associate Professor), Department of Forensic Medicine and Toxicology, Great Eastern Medical College, for giving valuable suggestions during the study period.

Conflict of Interest: None

Source of Funding: Self

Ethical Clearance: Taken from Ethical committee, Great Eastern Medical College.

References

1. V.V Pillay. Text book of Forensic Medicine and Toxicology, 16th edition, 2011, Paras medical Publishers, Postmortem changes, P: 112.
2. Somnath Das et al. Medical audit and death audit. JIAFM .2010; 32(4):369-370.
3. Rohina joshi et al. Chronic diseases now a leading cause of death in rural india – mortality data from the andhra pradesh rural health initiative. Int.J. Epidemiol . 2006 September; P: 1-8.
4. Gov of India. Central Bureau of Health Intelligence. National health profile 2018; Demographic indicators. 13th issue: 5.
5. Mohammed Murtuza Kauser et al. Study of mortality pattern in adults at a tertiary care teaching hospital in south India. RRJMHS .2014 october – December; 3(4):145-148.

Study of Adverse Drug Reactions (ADRs) Occurring with the Drug Use in a Tertiary Hospital

R. Selveraj

Associate Professor , Department of Pharmacology Kerala Medical College Hospital, Mangode, Cherpullassery, PalaKKad Dt, Kerala

Abstract

Aim: To study the adverse drug reactions (ADRs) occurring with the drug use in a tertiary care hospital.

Materials and Method: This observational study conducted over a period of four months (01-Nov-2015 to 29—Feb-2016). The Central Drug Standard Control Organization (CDSCO) suspect ADR forms (15) were distributed to all clinical departments personally in Kerala medical college hospital and research center, Mangode, Cherpullaserry. Regular visits were carried out twice a week for collecting data reports. They were then analyzed, compared with state data, national data, and international data. **Results** A total of 25 suspected Adverse Drug Reactions forms were reported during the period of four months of the study of the out patients and in patients departments of the hospital. Among all drugs NSAIDS, followed by Antimicrobials, Antipsychotics, Bronchodilators, Antihypertensive and oral hypoglycemic agent etc.. were reported to have adverse drug reactions. Most common route of adverse drug reactions was oral, followed by IV, IM, S/C, Topical and Inhalational routes. Reactions mostly seen affecting Skin, Gastrointestinal systems, Central nervous system, and Hematological system Most patients recovered from adverse events taking suitable measures like complete stopping the offending agent, or were prescribed antihistamines, steroids in addition **Conclusion:** Awareness about ADR reporting is still poor amongst healthcare professionals in India. The incidence and severity of Adverse Drug Reactions documented in our study are lower than those reported in other studies. NSAIDS comprise the major drug family associated with adverse drug reactions so should be rationally prescribed. Improved communication between the physicians and nurses with the pharmacovigillance centre in the hospital is suggested.

Key words: *Adverse drug reactions, CDSCO, Pharmacovigillance*

Introduction

According to WHO definition an Adverse Drug Reaction (ADR) is a response to a drug that is noxious and unintended, and occurs at doses normally used in human for the prophylaxis, diagnosis, and treatment of disease or modification of physiological function.[1]

Ultimately pharmacovigilance is concerned with identifying the hazards associated with pharmaceutical products and with minimizing the risk of any harm that may come to the patient.

The safety of prescription drugs represents a major public health concern. Adverse drug reactions are considered to be one of the leading causes of death among hospitalized patients.[2] Previous studies suggest that approximately 0.5% of all emergency department visits and tertiary care visits result from adverse drug reactions.

However, low- to middle income countries, which represents more than two thirds of the world population account for a tiny fraction of all the adverse drug reaction data. Among the reasons for this under-reporting are the difficulty that the patients and the providers may have liking a particular symptom or condition to a specific drug particularly for individuals who have chronic

Corresponding Author:
Abhay P.Betala
Assistant Professor, Department of Forensic Medicine
Vinayaka mission medical college, Karaikal, 609609,
Pondicherry email.id : apbetala@gmail.com

illness and are taking several medications, a common clinical scenario in tertiary care. Thus, there is a need to capture safety data for drugs in a country like India.

Documentation of Adverse Drug Reactions:

The significant adverse reaction of any drug should be notified within seven days. The other facts related to adverse events should be informed within eight days. [3] The drug reaction form can be collected at pharmacovigillance center.

The filled adverse drug reaction form can be submitted to the peripheral pharmacovigilance center. After reviewing the form, the center forwards it to the regional center and after that it is propelled to zonal center.[5,6,7]

This program is overseen by the central drugs standard control organization. (CDSCO).[8,9]

Patient reporting has been incorporated into pharmacovigillance systems in several countries including U.S.A, Canada, Australia, Newzeland Denmark, Sweden, and the Netherlands. Until very recently, however, patients in the U.K. were not able to report directly suspected Adverse Drug Reactions to Medicine and Health Care Regulatory Agency (MHRA), although some organizations has been proposing this for several years. In 2001, the UK Consumer Association called for patient reporting to be introduced after highlighting the fact that doctors were often failing to pass on information about suspected adverse reactions to drugs to MHRA. [10]

Aim of the study

To study the Adverse Drug Reactions occurring with the drug use in tertiary care hospital

Objective of the study

7. Critically evaluate the Adverse Drug Reactions occurring with the use of drugs in a tertiary care hospital over a period of 4 months (2015-16)

8. To establish the causality of Adverse Drug Reactions occurring with the use of drugs in the tertiary care hospital using the WHO causality assessment scale and the Naranjo causality assessment scale.

9. To compare the incidences of Adverse Drug Reactions occurring in tertiary hospital, with state data, and international (global) data.

Methodology

This observational study is to be conducted over a period of 4 months (01-Nov-2015 to 29—Feb-2016). Permission obtained from the Review and approval by the Institutional Ethics Committee to conduct the study. An introductory lecture is organized in the academic society of the institute to orient the clinicians towards pharmacovigilance and spontaneous reporting system. The Central Drug Standard Control Organization (CDSCO) suspect Adverse Drug Reactions forms (downloaded from CDSCO website) was distributed to all the clinical departments personally in Kerala medical college hospital and research center, Mangode, Cherpullaserry. . The forms contains the patient details, The description of the reactions, concomitant medication, coexisting illness, any rechallenge, dechallange etc.

On receiving information from the clinical departments, visit to the hospital and interact with the doctors to gather complete information on the Adverse Drug Reactions. The suspected Adverse Drug Reactions will be carefully analyzed and documented. Apart from this, regular visits will be carried out in respective departments and forms were collected twice in a week for analyzing the data and comparing the incidences of Adverse Drug Reactions occurring in a tertiary care hospital, with State data, national data and international (global) data

Results

Out of 25 cases reported with Adverse Drug Reactions in 4 months period, NSAIDS was found to be the most common implicating agent followed by Antimicrobials, Antipsychotics, Antacids, Vitamins and Minerals, Bronchodilators, Antihypertensive and oral hypoglycemic agent etc.. were reported to have adverse drug reactions.

Most Affected organ system was Skin, followed by Gastrointestinal systems, Central nervous system, and Hematological system.

According to Naranjo's Causality assessment scale(16) , 18 (72%) cases were Definite, 4 (16%) Probable and Possibly (Unrelated) 2 (4%) cases were reported as causing Adverse Drug Reactions.

As per the Hartwigs level of severity scale(13) 15 (60%) cases were found to have mild reaction and 5

(20%) cases each with moderate and severe reactions

According to Rawlins and Thomson(12) the type of reaction was classified as Type A (Predictable) with 21 (84%) cases and 4 (16%) cases with type B (Bizarre) reactions.

Looking for the Outcome of the patients, 15 (60%) cases were treated on OPD basis, and 10 (40%) cases required Hospitalization. Among them, 14 (56%) cases were recovered completely, 8 (32%) cases were in a state of recovering and 3 (12%) cases were continuing the treatment.

Most common route of adverse drug reactions was oral, followed by IV, IM, S/C, Topical and Inhalational routes.

Most patients recovered from adverse events taking suitable measures like complete stopping the offending agent, or were prescribed antihistamines and steroids.

Diagnosis, and drugs caused adverse drug reactions, route of administration

Discussion

This study tried to find out the pattern of adverse drug reactions of drugs used in tertiary care hospital. The number of reports we received were 25 out of 30747 cases treated, which amounted to an incidence of 0.081% in our set up. In comparison with the study for search of adverse drug reactions in hospital patients in Embase and Medline found the occurance of 2 – 27.7%, this can be considered as under reporting.[11] It is a universal problem and many reasons are identified such as busy schedule of clinicians, lack of knowledge about the exact authority to report adverse drug reactions to, lack of incentives, reporting process being tedious and inadequate expertise. Our verbal discussions with clinicians revealed similar reasons for underreporting in our institution.

The demographic details of our study showed female gender predominance over males, which was similar to that reported in other studies found in the literature. [12, 13] This might be due to higher emotion quotient in females which makes them more sensitive to the pharmacological actions of medicines.

The most common category associated with adverse drug reactions was dermatology (44%). This finding is concurrent with the study carried out by Coelho et al. (2002) and Rajesh et al. (2008), but it differs from reports of Suh et al. (2000), where gastrointestinal manifestations had the highest rate. Of the dermatological reactions observed in the hospital , itching were seen in 45.45 % and rashes in 36.36%

The incidence rate of NSAIDS adverse drug reactions in this study was found to be comparatively high when compared to other drugs.

Conclusion

The reporting rate appeared to be low so there is need for increasing knowledge and awareness. Educational interventions like conducting CME and training programmes can improve the knowledge towards pharmacovigilance. However monitoring adverse drug reactions is an ongoing and continuing process. Since newer and newer drugs hit the market the need for pharmacovigillance grows more than ever before. Imparting knowledge and awareness of adverse drug reactions reporting of health care professionals would introduce the reporting culture among medical practitioners and increase the reporting rates of adverse drug reactions. Careful considerations involved in planning and monitoring of drug therapy will lead to preventions of adverse drug reactions.

Acknowledgement: We wish to thank all our clinicians, nursing staff, emergency staff of K M C H for kind support in carrying out this study. Also, we extend our thanks to Chairman, K M C H, and Principal for their constant and valid guidance, support, and encouragement.

Ethical Clearance: Institutional ethical clearance was obtained

Conflict of Interest : Nil

Source of Funding : Nil

References

6. International drug monitoring : WHO Technical report. Technical report series No. 498. The role of national centres 1972. P 9. htpps://apps.who.int/iris/bitstream/handle/10665/40968/WHO_TRS_498.pdf
7. Brain L. Storm. Pharmacoepidemiology. Third edition – Willey online library. First published: 28-August-2000,Print-ISBN:9780471899259. https://onlinelibrary.wiley.com/doi/

book/10.1002/0470842555.

8. David W. Bates, MD, MSc; David J. Cullen, MD; Nan Laird, PhD; et al. Incidence of adverse drug events and potential drug events. JAMA.1995-Jul-5;274(1):75-6 https://www.ncbi.nlm.nih.gov/pubmed/7791255
9. David C Classen, MD, MS: Stanley L. Pestotnik, MS, R Ph; R Scott Evans, PhD; et al. January 22, 1997. Adverse Drug Events in Hospitalized Patients Excess Length of stay, Extra Costs, and Attributable Mortality. JAMA. 1997; 277(4):301-306. doi:10. 1001/jama.1997.03540280039031

https://jamanetwork.com/journals/jama/article-abstract/413536

10. Stephen A.Goldman MD. Limitations and strengths of spontaneous report data. Clinical therapeutics , 1998 – 4:40-44. Elsevier. https://doi.org/10.1016/SO149-2918(98)80007-6Get rights and content
11. Palaian S_, Mishra P_, Shankar PR_, Dubey AK, _ Bista D_, Almeida R_. Safety monitoring of drugs –where do we stand? Katmandu University Medical Journal (KUMJ) 01 Jan 2006, 4(1):119—127] (PMID: 18603884). https://europepmc.org/abstract/med/18603884
12. P Ravi Shankar, P Subish, P Mishra, AK Dubey. Teaching pharmacovigillance to medical students and doctors. Year : 2006 / Volume : 38 / Issue : 5 / Page : 316-319. http://www.ijp-online.com/article.asp?issn=0253-7613;year=2006;volume=38;issue=5;spage=316;epage=319;aulast=Shankar
13. Vikas Dhikav, Sindhu Singh, KS Anand. Adverse Drug Reaction Monitoring in India. Indian Academy of Clinical Medicine 5(1):27-33. January 2004. JIACM 2004;5(1):27-23 https://www.researchgate.net/publication/260152868_Adverse_Drug_Reaction_Monitoring_In_India.
14. Amrita. P, Singh SP, Status of spontaneous reporting of adverse drug reaction by physicians in Delhi. Indian Journal of Pharmacy Practice, 2011; 4(2):29-36. www.ijopp.org/article/230
15. Anonymous UK call for patient adverse drug reaction script 2001 2634:4 https:// www.google.com/search?q= Anonymous+UK+call+for+patient+adverse+drug+reaction+reporting+script+2001+2634:4&tbm=isch&source=univ&safe= active& sa= X&ved=2ahUKEwiy4pf M1ZDAhX46nM BHQjTBAYQsAR6BAgJEAE
16. Uppsala Monitoring Centre: UMC. https://www.who-umc.org/
17. Rawlins MD, Thomson JW (1977). Pathogenesis of adverse drug reactions. Textbook of adverse drug reactions oxford: oxford university press. Dec24,2014. http://www.oalib.com/references/15027661
18. Hartwig SC(1) , Siegel J, Schneider PJ. Preventability and seviarity assessment in reporting adverse drug reactions. Am J Hosp Pharm. 1992 Sep; 49(9):2229-32 https://www.ncbi.nlm.nih.gov/pubmed /1524068
19. Glossary of terms used in Pharmacovigilance. Glossary – UMC https://cdsco.gov.in/opencms/opencms/en/home/
20. Central Drugs Control Organization (CDSCO). Directorate of Helth Services, Ministry of Health and Family Welfare, Government of India. https://cdsco.gov.in/opencms/opencms/en/Home/
21. Naranjo CA, Busto U Sellers EM, Sandor P, Ruiz l, Roberts EA et al. a method for estimating the probability of adverse drug reactions. ClinPharmacol Ther. 1981 Aug;30(2)239-45 https//www.ncbi.nlm.nih.gov/pubmed/7249508
22. ICH Harmonized Tripariate Guideline. Step4 version dated 12 November 2003. https://www.ich.org/fileadmin/ Public_Web_Site/ICH_Products/Guidelines/Efficacy/E2D/Step4/E2D_Guideline.pdf

Pattern of Homicidal Deaths at Raichur District Region – A Retrospective Study

Ravishankar M G[1], Sunil Kumar Kainoor[2], Suraj[1], S Sharmila[3],

[1]Final year Postgraduate Student, [2]Assistant Professor, Department of Forensic Medicine and Toxicology, Raichur Institute of Medical Sciences, Raichur, Karnataka

Abstract

Background: It is a retrospective study carried out to find out the pattern of homicidal deaths in around the Raichur district region, among autopsies conducted at mortuary, RIMS, Raichur.

Materials and Methods: This is a two year retrospective study of autopsies conducted from January 2016 to December 2017 at Raichur Institute of Medical Sciences, Raichur. The objectives of the study were to know the pattern of homicidal deaths in and around Raichur district and to study the various socio-demographic factors influencing the homicidal deaths. Using a predefined and structured Performa, all the necessary details pertaining to the cases were collected from the inquest report and were analyzed.

Results: There were 757 total cases of autopsies conducted during the study period, of which there were 51 (6.73%) homicidal deaths. Male preponderance, 20-29 age group, blunt weapons - most commonly used, neck - commonest region of body involved constitutes 29.52%, most common cause of death - hemorrhage and shock 25.49%(13) equally followed by Head injury, maximum homicide took place at the victim‘s residence (58.82%), the most common motive behind the homicide was Infidelity (21.56%), maximum numbers of homicides were committed by Spouse (27.45%).

Conclusion: An attempt is made to know the socio demographic profile of the deceased so as to understand the sociological, economical, demographic and psychological aspects influencing homicidal deaths. The spurt in the homicidal deaths in our region may be attributed to the poor socio – economic condition, unemployment among young people, marital and family disputes, decreasing value based morality in the society, soft and sometimes toothless law enforcement agencies.

Key Words: *Retrospective study, Homicidal deaths, Socio demographic analysis.*

Introduction

Homicide means one human being causes death of another. Not all homicide is murder, as some killings are manslaughter, and some are lawful, such as when justified by an affirmative defense,

Like insanity or self defence.[1] A homicide is usually well-planned, therefore not normally witnessed. Killings remained the same v.i.z. lust for money, women and land. To commit murder, two elements (Mens–rea which means preplanning or afore thought and Actus Reus which means the actual execution).[2]

Homicide is defined as killing of one human being by another human being and is one of the leading causes of unnatural deaths.[3] Unlawful killing of human being is murder (S.300 IPC). Culpable homicide cases may be amounting to murder (S.299 IPC) or not amounting to murder (S.304 IPC). Punishment of murder (S.302IPC) is death or imprisonment for life and also fine. The various patterns of homicidal deaths include assault by sharp weapon, blunt weapon, firearm, strangulation, homicidal hanging, smothering, drowning, burns, poisoning etc. [4]

Corresponding Author:
Sunil Kumar Kainoor
Assistant Professor, Department of Forensic Medicine and Toxicology, Raichur Institute of Medical Sciences, Raichur, Karnataka-584102
Email- kainoor24@gmail.com, Contact- 9902178214

Material and Method

This 2 year retrospective study was analyzed among autopsies conducted from January 2016 to December 2017 at Raichur Institute of Medical Sciences, Raichur. The objectives of the study were to know the pattern of homicidal deaths in and around Raichur city and to study the various socio-demographic factors influencing the homicidal deaths. Using a predefined and structured Performa, all the necessary details pertaining to the cases were collected from the inquest report- which includes police enquiry report, witness statement and relative statement, findings of post-mortem examination report, hospital case sheet extracts, histo-pathological examination report, toxicological (chemical) analysis report, crime scene photographs and was then tabulated to Microsoft Excel sheet 2007 for analysis and results were explained in number of cases and percentage. Prior to the study, ethical committee clearance was obtained from Institutional Ethical committee.

Results

There were 757 total cases of autopsies conducted during the study period, of which there were 51 (6.73%) homicidal deaths. The maximum cases were observed among age group 20-29 years (31%), followed by 30-39 years (21%), 60+ years (13.72%), 40-49 years (11.76%), 50-59 years and 0-9 years (7.84%) . The least number of cases were observed among the age group 10-19 years (6%).

More number of deaths were observed among males i.e., 51% (n=26), on cross tabulating age group with gender wise distribution it was observed that more number of deaths among males (23.04%) was seen in the age group 20-29 years and 32% of female deaths in the age group 40-49 years. (Table 1)

Table 1: Age group v/s Gender distribution

Age group	Males	Females	Total	Percentage
0-9 yrs	1	3	4	7.8
10-19 yrs	3	3	6	11.8
20-29 yrs	6	4	10	19.6
30-39yrs	5	4	9	17.6
40-49 yrs	3	8	11	21.6
50-59 yrs	5	1	6	11.8
>60 yrs	3	2	5	9.8
Total	26	25	51	100

Most number of cases were observed during the winter season (42%) i.e., November to February (n=21) followed by summer season (33%) i.e., March to June and Monsoon (25%) i.e., July to October. Most deaths occurred instantaneously i.e., 58.82% (n=30), followed by within 12 hrs following assault (19.61%) and between 12-24 hours in 5.88% cases.

In our study the more number of deaths were due to assault by blunt force (37%), followed by asphyxial deaths (25% - which include throttling, smothering, drowning and poisoning cases), burn injuries (18%), no deaths due to firearm injuries were observed. (Table 2)

Table 2: Distribution of cases on types of injuries observed

Asphyxial deaths	**13**	**25%**
Firearm wound	0	0%
Stab wound	2	4%
Incised wound	4	8%
Chop wound	4	8%
Burns wound	9	18%
Blunt force wound	19	37%
Total	51	100%

Lungs are the most common organs to sustain injuries (33%), followed by brain (25%), no injuries to kidneys and spleen observed in our study. (Table 3)

Table 3: Distribution of cases on organs involved in injuries

Internal organs	Number of cases	Percentage
Lungs	17	33%
Brain	13	25%
Stomach and intestine	4	8%
Heart	2	4%
Spinal cord	2	4%
Testes	2	4%
Liver	1	2%
Multiple organs	1	2%
Kidney	0	0
Spleen	0	0
Misc	9	18%
Total	51	100%

Most number of assaults involved neck region (30%), followed by head and face (23%), multiple regions (21%) and least injuries on extremities (2%). (Table 4)

Table 4: Distribution of cases on region of body involved in injuries

Region of body	**Number of cases**	**Percentage**
Neck	15	30%
Head and face	12	23%
Multiple regions	11	21%
Abdomen	6	12%
Chest	3	6%
Chest and abdomen	1	2%
Extremities	1	2%
Others	2	4%
Total	51	100%

In our study more than half of the assault occurred in home (59%), the next most common was work place (20%) while 16% of assaults occurred in street.

In our study, a proper history or reason for homicide was uncertain in 13 cases (25%), followed by infidelity (22%), revenge (15%), heated arguments (14%) and 10% financial conflicts. (Table 5)

Table 5: Distribution of cases on motive before assault

	Number of cases	**Percentage**
Unknown	13	25%
Mental illness	1	2%
Dowry	2	4%
Property disputes	4	8%
Financial conflict	5	10%
Argument	7	14%
Revenge	8	15%
Infidelity	11	22%
Total	51	100%

Most number of assaults were by spouses (28%), followed by acquaintance (21%), relatives (21%), unknown assailants (14%) and parents and strangers in 8% cases each. Most common cause of death in our study is hemorrhage & shock and head injury each being 25%, followed by asphyxia (18%), septicemia (12%) and hypovolemic shock in 8% cases.

Discussion

In our study duration, (2 yrs) there were totally 757 cases autopsied of these 51 cases (6.73%) are homicidal deaths. In a similar study by Courtnee clark et al[5] with study duration being 5 years the total percentage of homicidal deaths are 5.32%. Similar studies by others

showed lesser proportion of homicidal deaths i.e., Shailesh Jhaveri et al[6] - 2.34 % (3 yrs), Dr. Basappa S. Hugar et al [2] 4.32% (3yrs) and Ashok K. Rastogi et al [7]– 4.25% (1 yr).

Majority victims in our study were males 50.98%, the results are in similarity with other studies conducted by Dr. Basappa S. Hugar et al [2] , Shailesh Jhaveri et al[6], Courtnee clark et al[5] and Ashok K. Rastogi et al [7].

The most common age group to suffer in our study is 20-29 years (31.37 %), the same results are observed in various other authors like Dr. Basappa S. Hugar et al [2] , Shailesh Jhaveri et al[6], Courtnee clark et al[5] except Ashok K. Rastogi et al[7] - 18-40 yrs.

In our study most cases are reported during the winter season 41.17% i.e., November to February (Nov-Feb), while in most other similar studies by Dr. Basappa S. Hugar et al [2], Shailesh Jhaveri et al[6], Courtnee clark et al[5] and Ashok K. Rastogi et al [7] the most number of cases are reported during warmer climate (Summer). Warren et al. (1981), who found homicide to have a seasonal pattern that changes from year to year. That is, a "peak month" in some years is a "trough month" in other years. The authors conclude that homicide is seasonal, but inconsistent[8]

Blunt weapons being the most common weapon used in our study (37.25%) and the same results observed by Shailesh Jhaveri et al[6], Courtnee clark et al[5] and Ashok K. Rastogi et al [7] while study by Dr. Basappa S. Hugar et al[2] the sharp weapons are the most common weapons of offence.

In our study the most common region of body involved sustaining injuries is neck (29.52%), the same results observed in other studies by Dr. Basappa S. Hugar et al [2], Courtnee clark et al[5] and Ashok K. Rastogi et al [7]. While in a study by Shailesh Jhaveri et al [6] multiple body structures involvement was common.

In our study both Shock and Hemorrhage (25%) & head injury (25%) are the most common cause of death, similarly in a study by Ashok K. Rastogi et al [7] shock and hemorrhage 46.34% followed by asphyxia 20.73% was observed. In another study by Shailesh Jhaveri et al [6] head injury (26.42%) is the most common cause of death.

Victim residence is the commonest site of occurrence of crime in our study (59%), the same observation was made by Shailesh Jhaveri et al[6], Courtnee clark et al[5] and Ashok K.Rastogi et al [7].

Conclusion

The present study pattern of homicidal deaths in the Raichur district region has provided a number of revealing information about homicidal deaths. There has been a steady increasing trend in the homicidal deaths in our area. The spurt in the homicidal deaths may be attributed to the poor socio – economic condition, unemployment among young people, marital and family disputes, decreasing value based morality in the society, soft and sometimes toothless law enforcement agencies.

The government and society should identify the various social, economical, moral and law enforcement agencies problems that are directly or indirectly leading to the rise in the incidence of homicidal deaths and should address it through proper agency or department.

Conflict of Intrest – None

Sources of Funding – Self

Ethical Clearence – Institutional Ethical Clearence Taken

Refernces

1. Cornell Law University. Legal information Institute. (Online). Available at http//www.law.cornell.edu/wex/homicide. Accessed on May 2019
2. Hugar BS, Chandra GYP, Harish S, Jayanth SH. Pattern of Homicidal Deaths. JIAFM: 2010; 32(3):194-98.
3. Reddy KSN. The Essentials of Forensic Medicine and Toxicology. 29th ed. Hyderabad: Medical Book Company; 2010. 259 - 60.
4. Parikh C.K. Parikh's Text Book of Medical jurisprudence, Forensic Medicine and Toxicology for Classrooms and Courtrooms. 6th ed. New Delhi: CBC Publishers and Distributors;1990: 4.23pp.
5. Clark C, Mole CG, Heyns M. Patterns of blunt force homicide in the West Metropole of the City of Cape Town, South Africa. S Afr J Sci. 2017;113(5/6).
6. Jhaveri S, Raloti S, Patel R, Brahbhatt J, Kaushik V. Profile Of Homicidal Deaths: A Three Year Study at Surat Municipal Institute Of Medical Education And Research, Surat. 2011 -13, NJCM; 2014; 5(4):406-9

7. Rastogi AK, Singh BK, Dadu SK, Thakur PS, Lanjewar AK, Raput PP. Trends of Homicidal Deaths in Indore (M.P.) Region One Year Retrospective Study. JIAFM: 2013; 35(4):343-5.
8. Gould W, Coldren JD. Is crime seasonal? Illinois criminal justice information authority. Carolyn Rebecca Block Statistical Analysis Center. State of Illinois. January 1984. P 3.

Assessment of Serum Levels of Salusin α and Salusin β in Cardiovascular Disease Patients Undergoing Transcatheter Therapy

Ayat Saeed Awad*[1], Hanaa Addai Ali[2] , Mohauman Mohammad AL-Rufaie [3]

[1]Post graduate Student, College of Science, University of Kufa, Iraq, [2]Prof. College of Science, University of Kufa, Iraq, [3]Prof. College of Science, University of Kufa, Iraq

Abstract

Cardiovascular disease (CVD) covers some disorders, such as the diseases of the cardiac muscles and the vascular system supplying the heart, brain and extra vital organs. CVD morbidity and mortality is mainly due to coronary heart disease and cerebro-vascular diseases. The aims of the present is to measure the levels of Salusin α, Salusin β, lipid profile, fasting blood sugar, insulin, insulin resistance and HOMA IR in the serums of cardiovascular patients who are subject to catheterization and to compare these levels with the ones of the healthy group and their correlation with Salusins. The results have made use of the appropriate statistical methods.

The results of the research have shown that there is a significant increase in the salusin-β in CVD patients compared with that of the healthy group but Salusin α ($p>0.001$) witnessed a significantly lower level. The present study has demonstrated that VLDL.C, HDL.C, TG.C and Salusin-β are in a significant correlation with Salusin α. Also, Age, BMI, HOMA-β, Insulin, HOMA-IR, FBG, CHO and LDL.C have no significant correlation with Salusin α. The results have shown that BMI and Salusin α levels are significantly correlated with salusin-β. In addition, Age, HOMA-β, Insulin, HOMA-IR, FBG, CHO, LDL.C, VLDL.C, HDL.C and TG.C have no significant correlation with Salusin-β. BMI and HDL.C have shown a positive correlation with Salusin α. Moreover, Age, Insulin, HOMA-IR, HOMA-β, FBG ,CHO, LDL.C, VLDL.C and TG.C have shown a positive correlation with Salusin-β.

Key words: *Cardiovascular disease, Salusin α, Salusin β.*

Introduction

Cardiovascular disease (CVD) covers disorders like the cardiac muscles and the vascular system supplying the heart, brain and extra vital organs (1). CVD morbidity and mortality is mainly due to coronary heart disease and cerebro-vascular diseases (2). CVD is as a rule connected with atherosclerosis; an inflammatory disease characterized via the accumulation of lipids and fibrous elements in relation to medium arteries (3).

Salusins are taken as a category of bioactive peptides discovered through bioinformatics analyses of a complete length CDNA library. Recently, two associated peptides of 28 and 20 amino acids are renowned and characterized; they specified Salusin-α and Salusin-β. These peptides are believed to be biosynthesized of pre- prosalusin; an alternative-splicing production of the torsion dystonia–related gene (TOR2A), subsequent to frame shift reading and digestion at dibasic amino acids [(4)].

Salusins are synthesized ubiquitously in human tissues, counting the vasculature, central nervous system and the kidney. Salusin-α is present inside human plasma and urine[(5)]. Salusin-β quickly induces hypotension, bradycardia, and cardiac dysfunction during a cholinergic mechanism [(6)]. Salusin- β also stimulates human macrophage foam cell formation [(7)], proliferation of vascular smooth muscle cells and fibroblasts [(4)] and cardiomyocyte growth and anti-apoptosis [(8;9)].

Material and Method

A case control study is designed for a total of 60 subjects (44 males and 16 females, aged between 35 to

65 years) who consecutively registered in this study. The subjects are 30 Iraqi patients with cardio vascular disease (CVD). They have participated in the current study (8 female and 22 males). These patients are registered as CVD patients in the "open heart Unit" at "AL-Sader Teaching Hospital" in Najaf, Iraq. The patients' serum in the pre - and post-cardiac catheterization is collected and compared with that of the control group. Thirty healthy adults are selected as the control group. The range of their ages is analogous to that of the patients (35-65) years. Subjects who suffer from the apparent diabetes mellitus, acute infections, chronic liver diseases, renal disorders, cancers and patients with surgical procedures in the last 3 months, and nonsmoker are all excluded.

Five milliliters of venous blood after 12 hours fasting are drawn from the CVD patients and the healthy group during (8:30-10 A.M) from antecubital venipuncture using G 23 needles.

Hypertension is diagnosed as a systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg. The BMI is calculated as the ratio of weight (Kg) to height squared (m^2), by unit kg/m^2. Fasting analysis of serum glucose, lipid profile (CHO, TG.C, LDLC, and HDL.C) levels are measured by colorimetric method for the quantitative *in vitro* diagnostic measurement using kit (BIOLABO (France)). The Salusin α and Salusin-β are also measured using the Competitive-ELISA principle (Elabscince (USA)).The concentrations of Insulin serum are determined by ELISA kits (Calbiotech (USA)). Insulin resistance index (Homeostatic model assessment-insulin resistance,HOMA-IR) is estimated as follows: HOMA- IR = [glucose (in mg/dL) * insulin (μU/ml)] / 405.HOMA -β =360× Insulin /(Glucose-63) % [10].

Bio-statistical Analysis

The results are subjected to statistical analysis and are analyzed using Microsoft Excel 2013 and SPSS-20 (statistical package for social science-version 20). The results are expressed as numbers and as mean ± SD (Standard deviation). The significance of difference is assessed using paired t-test for two dependent means. The correlation of parameters is determined using Pearson's correlation coefficient, taking $p \leq 0.05$ as the lowest limit of significance [11].

The one-way ANOVA (Analysis of variance) and Fishers Least Significant Difference (LSD) are applied to compare the differences among the studied groups.

Results and Discussion

Clinical characteristics of the Studied Groups

The patients with cardiovascular diseases have shown a significantly higher level of fasting blood sugar (by definition, ($p<0.001$), higher level of insulin($p<0.001$), respectively), higher level of HOMA IR($p<0.001$), Lipid profile ($p<0.001$) and Salusin-β ($p<0.001$) in CVD patients (Group2), compared with the healthy group (Group1). Additionally, lower Salusinα and HOMA-β ($p>0.001$) are also seen, table (1).

Table (1) shows significantly higher levels in the Insulin, FBG and HOMA IR ($p<0.001$), except HOMA-β which records a non significant level in pre-catheterization (Group2), compared with post-catheterization (Group3). No significant difference is registered regarding salusin-β and insulin ($p>0.001$)

But there is a significant increase in FBG, HOMA IR, HOMA-β,Lipid profile, Salusinα, when the control (Group1) is compared with post-Catheterization (Group3).

The demographics and laboratory data of the Salusin α and Salusin β groups are summarised in tables (2) and (3). The levels of VLDL.C, HDL.C, TG.C and Salusin β are significantly higher with Salusin α for all ($p<0.05$)($p=0.036$) ($p=0.022$) ($p=0.035$), ($p=0.048$), respectively.

There is no significance correlation of Age, BMI, HOMA-β, Insulin, HOMA-IR, FBG, CHO and LDL with Salusin α ($p>0.001$), as in table (2).

BMI and Salusin α levels are significantly elevated with Salusin-β ($p<0.05$) ($p=0.033$) ($p= 0.048$), respectively. Moreover, Age, HOMA-β, Insulin, HOMA-IR, FBG, CHO, LDL.C, VLDL.C, HDL.C and TG.C have

no significant correlation with Salusin-β($p>0.001$), as in table (3).

This study has found that HDL.C is positively correlated with Salusin α. In addition, Age, BMI, Insulin, HOMA-β, HOMA-IR, FBG, CHO, LDL.C, VLDL.C, TG.C and Salusin-β showed a negative correlation with Salusin α, as in table (2).

Furthermore, HDL.C and Salusin α showed a negative correlation with salusin-β. Also, Age, BMI, Insulin, HOMA-IR, HOMA-β, FBG ,CHO, LDL.C, VLDL.C and TG.C showed a positive correlation with Salusin-β., as seen in table (3).

CVD is as a rule connected with atherosclerosis (3). Atherosclerosis is a chronic vascular disease, in which the arteries are thicken and lose their flexibility as a result of cholesterol sedimentation in the artery wall. In the early stages of the disease, cholesterol accumulates in arterial macrophages, alter them to lipid-loaded bubbles cells. Wide atherosclerosis narrows the artery lumen to reduce blood flow, and the can enhance the complete blockage of the artery (12; 13). Atherosclerosis occurs due to a variety of reasons, the most significant of which is the deposition of large amounts of cholesterol and calcium in the blood. It may happen because of obesity overcharged as a result of absence of exercise. Additionally, the increased blood pressure raises the risk of developing atherosclerosis (14; 15).

Table (1) Biochemical characteristics of CVD patients and healthy as control group

Parameters	Control group1 Mean±SD	Pre-catheter group2 Mean±SD	Post-cathetergroup3 Mean±SD	P value
Age	28.01±1.34	29.11± 1.27	29.11± 1.27	a) NS b) NS c) NS
BMI	26.32 ± 3.329	31.46 ±5.010	31.46 ±5.010	a) 0.001 b) 0.001 c) 0.001
SBP (mmHg)	122.6±1	145±2	139.5±2	a) <0.0001 b) <0.0001 c) NS
DBP (mmHg)	70.7±2	76.3±2	71.2±2	a) NS b) NS c) NS
FBG (mg/dl)	99.29 ± 8.52	166.62±35.24	143.87±36.17	a) 0.000** b) 0.001 c)0.019
Insulin(µlu/ ml)	10.67±3.30	19.43± 10.85	8.94± 4.05	a)0.000** b) NS c) 0.000**
HOMA IR	2.59 ±0.82	8.03 ± 4.85	3.33 ± 2.21	a)0.000** b) 0.001 c) 0.000**
HOMA-β	111.42±43.88	145.58±369.51	45.42±24.85	a)0.617NS b) 0.001 c) 0.151 NS
HDL.C	55.61±6.33	37.43 ±8.41	39.07±6.97	a)0.000** b) 0.001 c) 0.419 NS
VLDL.C	20.23±3.31	51.48±16.86	39.79±13.28	a)0.000** b) 0.001 c) 0.005**
LDL.C	86.00±14.95	185.48±45.20	165.93±45.37	a)0.000** b) 0.001 c) 0.106 NS

Cont... **Table (1) Biochemical characteristics of CVD patients and healthy as control group**

CHO	161.85±15.41	274.54±43.68	244.80±42.91	a)0.000** b) 0.001 c) 0.011
TG.C	101.28±16.99	258.02±84.39	198.95±65.81	a)0.000** b)0.001 c) 0.004**
Salusinα	11.00± 1.68	10.59± 1.35	13.10 ±1.85	a) 0.303 NS b) NS c) 0.000**
Salusinβ	11.87± 1.38	14.63± 1.10	10.86 ± 2.21	a) 0.03 b) 0.001 c) 0.001

a) Significant difference between values in Group(1) and Group (2), **b)** Significant difference between values in Group (3) and Group (1), **c)** Significant difference between values in Group (3) and Group (2), **=significant differences at 1%, NS =non-significant at the 0.05 level, FBG: fasting blood glucose, HOMA-IR: Homoeostasis model assessment-insulin resistance.Salusin-α has a mild hypotensive effect (4) and suppresses human foam cell formation via the down-regulation of acyl-CoA : cholesterol acyltransferase-1 (ACAT-1), which stores cholesterol ester changed from free cholesterol in macrophages. In previous studies, Serum salusin-α levels are significantly decreased in acute coronary syndrome (ACS) patients as compared with healthy people and are less in accordance with the severity of coronary atherosclerotic lesions amongst ACS patients. In coronary atherosclerotic lesions of ACS patients, the level of expression of Salusin-α is lower than that of Salusin- β (7).

Salusins essentially affect the cardiovascular system (6;7). Salusin-β is the most hypotensive peptide, its infusion is rapid and profoundly decreases blood pressure and heart rate. Moreover, it is demonstrated to cause cardiac dysfunction through a cholinergic mechanism in rats (6;8). It may have significant roles in myocardial growth and hypertrophy. Salusin-α and salusin-β show converse actions on atherosclerosis due to their opposite regulatory effects on acyl-coenzyme A: cholesterol acyltransferases-1 (ACAT-1). Both of the formation of macrophage foam cells and the enlargement of atherosclerosis are suppressed via Salusin-α. Serum Salusin-α levels are also reported to be significantly lower in patients with coronary artery disease and hypertensive patients where their Salusin-α level is inversely associated with carotid atherosclerosis (7;16).

Table(2) The relevance of Salusin α with concentrations of biochemical parameters in the patients group

Variables	r	P
Age	-0.000	1.000
BMI	-0.304	0.109
FBG	-0.164	0.394
Insulin(μIU/ml)	-0.179	0.353
HOMA-IR	-0.227	0.237
HOMA-β	-0.077	0.693
CHO	-0.194	0.314
LDL.C	-0.119	0.538
VLDL.C	-0.392*	0.036
HDL.C	0.425	0.022
TG.C	-0.394*	0.035
Salusin β(pg/ml)	0.371*	0.048

P- Value ≤ 0.05 = significant, r : Pearson correlation

Table(3) The relevance of Salusin β with concentrations of biochemical parameters in the patients group

Variables	r	P
Age	0.182	0.345
BMI	0.397*	0.033
FBG	0.122	0.527
Insulin(μIU/ml)	0.127	0.511
HOMA-IR	0.102	0.597
HOMA-β	0.225	0.240
CHO	0.038	0.844
LDL.C	0.118	0.542
VLDL.C	0.150	0.438
HDL.C	-0.125	0.517
TG.C	0.149	0.441
Salusin α (pg/ml)	-0.371*	0.048

P- Value ≤ 0.05 = significant, r : Pearson correlation

Angiotensin II is associated with the genesis of arterial hypertension and cardiovascular remodeling (17;18). Renin-angiotensin system intervention in hypertensive patients has shown lower morbidity and mortality (19; 20). Salusin-β gene silence has normalized the increased circulating Ang II levels in addition to the local An II contents in both myocardium and mesenteric artery in spontaneously hypertensive rats (SHR). Additionally, the up-regulation of AT receptors within myocardium and mesenteric artery in SHR (4) are inhibited through the knockdown of Salusin-β.

The inhibitory effect of Salusin-β on the activation of angiotensin system may partially contribute to the attenuation of hypertension and cardiovascular remodeling. It is well known that increased oxidative stress is associated with endothelial dysfunction, apoptosis, hypertrophy, inflammation, fibrosis and cell migration relative to vascular remodeling of hypertension (21;22).

Conclusion

In conclusion, our results suggest that Salusin-α and Salusin-β prove contrasting effects on atherosclerosis and that Salusin-α and Salusin-β possess anti-atherogenesis and proatherogenesis, respectively.

The current study has concluded that in patients with CVD, Salusin β levels have recorded a significant increase in the serum of CVD.

Further, Lipid Profile changes are directed by the age of patients in CVD.

Salusinα increase in serum of post catheterization and Salusin β decrease in serum of post catheterization.

In order to improve diagnosis and treatment of CAD, the research community needs to understood how the immune response is analogous and how it differs in men and women with atherosclerosis.

Acknowledgement: I would like to thank my Supervisor Dr. Hanaa Addai Ali for her helpful comments and assistance with this study.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1) Mendis, S., Puska, P. & Norrving, B.” Global Atlas on Cardiovascular disease prevention and control”, World Health Organisation in collaboration with World Heart Federation and World Stroke organisation, Geneva Switzerland.2011.

2) Sanders, T. & Emery, P. “Molecular basis of human nutrition”, Taylor and Francis, London.2003.

3) Lusis, A.J. “Atherosclerosis”,2000; Nature, vol. 407, no. 6801, pp. 233-241.

4) Shichiri M, Ishimaru S, Ota T, Nishikawa T, Isogai T,Hirata Y: “Salusins: newly identified bioactive peptides with hemodynamic and mitogenic activities”. Nat Med .2003; 9:1166–1172.

5) Sato K, Koyama T, Tateno T, Hirata Y, Shichiri M:” Presence of immunoreactive salusin-α in human

serum and urin"e. Peptides. 2006; 27: 2561–2566.

6) Izumiyama H, Tanaka H, Egi K, Sunamori M, Hirata Y,Shichiri M: "Synthetic salusins as cardiac depressors in rat".Hypertension .2005; 45: 419–425.

7) Watanabe T, Koba S, Shichiri M, et al: "Impact of salusin-α and -β on human macrophage foam cell formation and coronary atherosclerosis". Circulation .2008; 117: 638–648.

8) Yu F, Zhao J, Yang J, et al:" Salusins promote cardiomyocyte growth but does not affect cardiac function in rats". Regul Pept .2004; 122: 191–197.

9) Hong-Xiao Y, Li L, Yan-Xia P, et al:" Salusins protect neonatal rat cardiomyocytes from serum deprivation-induced cell death through upregulation of GRP".78. J Cardiovasc Pharmacol .2006; 48: 41–46.

10) Aravind T, Poornima S S, Sibasis S A and Kumar" Prevalence of insulin resistance: a prospective study in North Indian population". 2005;India*n J Clin Biochem*. 20, 10-17.

11) Daniel W ."Basic concepts and methodology for the Health sciences". *Biostatistics*. 9th Edition, John Wiley and Sons Inc:2010; 170-400.

12) Ross, R.: "The pathogenesis of atherosclerosis". 1993; Nature 362,801–809 .

13) Glass, C.K., Witztum, J.L.: Atherosclerosis: the road ahead. 2001;Cell 104, 503–516 .

14) Bergounioux ."Mathematical Image Processing " , University of Orl´eans, France , 2010.

15) K. Hansson ," Inflammation, atherosclerosis,and Coronary Artery Disease" , The new england journal , 2005.

16) Ti Y, Wang F, Wang ZH, Wang XL, Zhang W, Zhang Y, et al. "Associations of serum salusin-alpha levels with atherosclerosis and left ventricular diastolic dysfunction in essential hypertension". J Hum Hypertens.2012; 26: 603-9.

17) Kwon, W. Y. *et al.*"Interleukin-10 deficiency aggravates angiotensin II-induced cardiac remodeling in mice".2016;*Life Sci.* **146,** 214–221 .

18) Deng, Y. *et al.*"Prenatal inflammation-induced NF-kappaB dyshomeostasis contributes to renin-angiotensin system over-activity resulting in renatally programmed hypertension in offspring".2016;*Sci. Rep*. **6,** 21692 .

19) Munoz-Durango, N. *et al.* "Role of the Renin-Angiotensin-Aldosterone System beyond Blood Pressure Regulation: Molecular and Cellular Mechanisms Involved in End-Organ Damage during Arterial Hypertension". 2016;*Int. J. Mol. Sci.* **17,** 797 .

20) Te, R. L., van Esch, J. H., Roks, A. J., van den Meiracker, A. H. & Danser, A. H. "Hypertension: renin-angiotensin-aldosterone system alterations". 2015;*Circ. Res.* **116,** 960–975 .

21) Sinha, N. & Dabla, P. K." Oxidative stress and antioxidants in hypertension-a current review". *Curr. Hypertens.2015*; *Rev*. **11,** 132–142 .

22) Cheng, P. W. *et al."*Resveratrol Inhibition of Rac1-Derived Reactive Oxygen Species by AMPK Decreases Blood Pressure in a Fructose-Induced Rat Model of Hypertension". 2016*;Sci. Rep*. **6,** 25342

Effect of Multi-Wall Carbon Nanotubes on the Microhardness of the Tooth Enamel

Mohammed K. Jawad[1], Abbas F. Ali[2], Ali B. Roomi[3]

[1]Specialized Dental Center Sumer, Thi-Qar Health Directorate, Thi-Qar, Iraq, [2]College of Dentistry at The University of Baghdad, Baghdad, Iraq, [3]College of Health and Medical Technology, University of Al-Ayen, Thi-Qar, Iraq.

Abstract

Background: The object of this study was to investigate the effect of addition of Multi-Wall Carbon Nano Tubes (MWCNTs) in different concentration (0.001g/20ml, 0.005g/20ml , 0.01g/20ml and 0.02g/20ml) in (dimethyl sulphoxide) to tooth enamel. It is intended to evaluate enamel hardness in (Kg .m-2) before and after the addition of (MWCNTs).

Materials and Method: Thirty specimens were prepared for this study, to measure the hardness of the enamel.

Results: The results enamel hardness after MWCNTs application With DMSO showed a significant increase ($P<0.01$) between groups of 0.001g/20ml , 0.005 g/20ml , 0.01 g/20ml and , 0.02 g/20ml concentration compared with control group. Where the highest mean value obtained was in 0.02g/20ml concentration which had a mean value of 527.18 ± 2.904 Kg $.m^{-2}$, while the lowest mean value was for control group which had a mean value of 334.87± 2.904 Kg $.m^{-2}$. Polished enamel surface groups showed a significant increase 452.59 ± 1.84 Kg $.m^{-2}$ ($P<0.01$) in hardness when compared with unpolished enamel surface groups 430.16 ± 1.84 Kg $.m^{-2}$ ($P<0.01$).

Conclusion: The highest mean value obtained was in 0.02g/20ml concentration in the hardness suspended in DMSO. The results showed a significant increase in the hardness for polished enamel surface samples compared with unpolished enamel surface samples in DMSO.

Key words: *Multi-Wall Carbon NanoTubes (MWCNTs), enamel hardness*

Introduction

Enamel, the outer hard tissue layer of tooth crowns, is a composite material that comparable to other biological tissues like bone or dentin exhibits a unique and complex hierarchical structure (1). The bulk of human teeth consists of two main mineralized tissues, collagen-rich dentine and highly mineralised enamel. They join forming a complex and mechanically durable dentine–enamel junction (DEJ) that contributes to the lifelong success of the tooth structure under thermo-mechanical loadings encountered in the oral cavity under the conditions such as mastication, chemically active environment and thermal shock (2, 3).

Enamel is the hardest tissue in the human body and is considered a nanostructured biocomposite in which its mineral phase predominates (95-96 wt. %) (4).

In this mineral portion, large hexagonal carbonated hydroxyapatite crystals are tightly packed creating prisms with a keyhole-like structure of about 5 μm in diameter (5). Prisms are aligned and run approximately perpendicular from the dentin-enamel junction to the tooth surface (3). Each prism is separated from each other by a nanometer-thin layer of a protein-based organic matrix (6). The term “Nano” is derived from the Greek word “dwarf ”. More simply speaking, one nanometer is one-billionth or 10-9 of a meter (7-9). Nanotechnology can be classified in terms of application in three broad and extensively overlapping categories (10):

- Nanoelectronics
- Nanomaterials/particles
- Nano-biotechnology

Carbon nanotubes (CNT) are a new crystalline form of carbon. Wound in a hexagonal network of carbon atoms constituting a graphene nanofoil, these hollow cylinders can have diameters as small as 0.7 nm with lengths that can range from a few micrometres, and reach several millimeters in length (11). Each end can be opened or closed by a fullerene half molecule. These nanotubes can have a single layer (SWCNT for single walled carbon nanotube) or several layers (MWCNTs for multi walled carbon nanotube) of coaxial cylinders of increasing diameters in a common axis. Multilayer carbon nanotubes can reach diameters of 100 nm (12).

Enamel surface microhardness refers to a tooth's resistance to scratching, abrasion, and indentation. A substantial number of mineral ions can be removed from hydroxyapatite latticework without destroying its structural integrity; however, such demineralized enamel transmits hot, cold, pressure and pain much more readily than normal enamel. Microhardness tests are commonly used to study the physical properties of materials, and they are widely used to measure the hardness of teeth (13, 14). Knoop (KHN) and Vicker (VHN) hardnesses have reported approximately the same value (15). The average hardness value for enamel and dentin is in the range from 270 to 350 Knoop microhardness or from 250 to 360 Vickers microhardness and from 50 to 70 Knoop microhardness respectively (16).

Materials and Method

Functionalisation of Commercial Carbon Nano Tubes (17). One gram MWCNTs was transferred into a mixture of nitric acid (10 cm3) and sulphuric acid (30cm3). This mixture was then heated at 50 ◦C for 24 hrs after which the MWCNTs were filtered off using Nylon filter paper (pore size 0.45 micrometer). This was followed by subsequent washing with distilled water until the pH was almost neutral. The MWCNTs were then dried under vacuum at room temperature. Then dried in furnace oven for 2hr.In the third step 0.02 g from MWCNTs were put in 20 ml DMSO. The MWCNTs is mixed with DMSO. The whole solution is transferred into sonicator. Twenty four sample in DMSO in different concentration for micro hardness test. The samples should be kept in a water. These samples were shaken in the vibrator for limited period extend 10min for 3 time in 6 continuous days.

Grouping of Samples

The group contains 30 samples to measure the hardness of the enamel. Samples were collected from healthy teeth of patients attending a dental teaching hospital at the University of Baghdad collage of Dentistry, also Thi-Qar specialized dental center in department of Orthodontics of the ages ranging between 15 - 24 years. The first selection criterion for the sample was tooth quality. Only teeth with no visible defects were selected, not taking into account any damage at the micro structural level. They were without any caries, no attrition or erosion. The patients were non-smokers and do not consume alcoholic beverages. The second selection criterion was that the teeth belonged to mandibular first premolar.

The Hardness of a material

The hardness of a material its resistance to penetration under a localized pressure or resistance to abrasion. The baseline of the hardness of base lines was measured through the use of Micro -Vickers Hardness Testing Machine (CV-400 DM, Europe) (Figure1), with a load of 500 g and 1000 g, in 5 seconds.

Principle of Hardness Determination

The micro hardness test involves a microscopic and static method, of which the results are mostly expressed in terms of Vickers and Knoop hardness numbers. The micro hardness tester is provided with an optical magnifying system. The hardness is determined by penetrating a diamond pyramid indenter under a known test force into the surface of test piece and then measuring the diagonal of the indentation left on the surface after removal of the test force.

The hardness number is calculated upon the below equations.

$$HV = 1854\ 4F/d$$

Vickers Test

HV: Vickers hardness number, in kg f. mm-2, F : Test force, in kg f, d :Diagonal length of the indentation, in mm2.

Sample Pr eparation to Measure The Hardness

The total number of samples were 30 samples to measure the hardness of the enamel, and divided the group to subgroups upon the following design.

Control group : (3 unpolished enamel surface and 3 polished enamel surface *samples).*

Group (0.001g/20ml) : (3 unpolished enamel surface and 3 polished enamel surface samples)

Group (0.005g/20ml) : (3 unpolished enamel surface and 3 polished enamel surface samples)

Group (0.01g/20ml) : (3 unpolished enamel surface 3 polished enamel surface samples)

Group (0.02g/20ml) *:* (3 unpolished enamel surface and 3 polished enamel surface samples) *Groups (*0.001g/20ml, 0.005g/20ml*,* 0.01g/20ml*,* 0.02g/20ml) *treated with MWCNTs, the special area on the middle third of the labial enamel surface were chosen 5mm away about the cusp tip and whit a line representing axis (X) for all teeth. All have samples examined for the micro hardness. .*

Statistical Analysis

Statistical analysis was done using the software SPSS version 17.0; the results were expressed as mean ± standard deviations (mean ± SE). One way ANOVA was used to compare parameters in different studied groups. P-values ($P < 0.01$) were considered statistically significant.

Results

Statistical Analysis of the results was used to evaluate , enamel hardness in (Kg .m-2) after MWCNTs application with DMSO in different concentration treatment.

Enamel Hardness Test

Control group comport with groups dealing with MWCNTs application with DMSO in different concentration treatment and different surfaces treatment.

MWCNTs application with DMSO in different concentration treatment and different surfaces treatment

Table (1) showed that the highest mean value found in 0.02g/20ml concentration which has a mean value of 527.18 ± 2.904 Kg .m-2 , while the lowest mean value was for the control group which has a mean value of 334.87 ± 2.904 Kg .m-2 . The results of LSD test showed a statistically significant differences between groups in different concentration treatment as in the figure (2). And also table (1) showed that the highest mean value represent in polished enamel surfaces which has a mean value of 452.59 ± 1.84 Kg .m-2 , while the lowest mean value was for in unpolished enamel surfaces which has a mean value of 430.16 ± 1.84 Kg .m-2 . The results of LSD test showed a statistically significant differences between groups in different surfaces treatment as in the figure (3).

Table (1) showed that the highest mean value present a concentration of 0.02g/20ml in polished surfaces which has a mean value of 547.07 Kg .m-2 , while the lowest mean value was for the control group in unpolished surfaces which has a mean value of 330.30 Kg .m-2 and the results of LSD test showed a statistically significant differences between groups in different concentration treatment and different surfaces treatment.

Discussion

Micro Hardness of Dental Enamel

Based on the findings of the current study, the average value of Vickers enamel microhardness was 334.87 ± 2.91, which is similar to the findings of Panich and Poolthong (18) , enamel hardness depends on different factors such as degree of enamel mineralization, enamel prisms and enamel tufts variations in different areas of enamel, presence or absence of any structural defects in the enamel, type of the teeth (whether it is anterior or posterior), and procedures for preparing the samples to perform the hardness test (13). Other factors influencing enamel hardness are the bio environmental factors, fluoridation of the drinking water, age of the teeth, and different eating habits in different societies (19).

Enamel Hardness After MWCNTs Application With DMSO

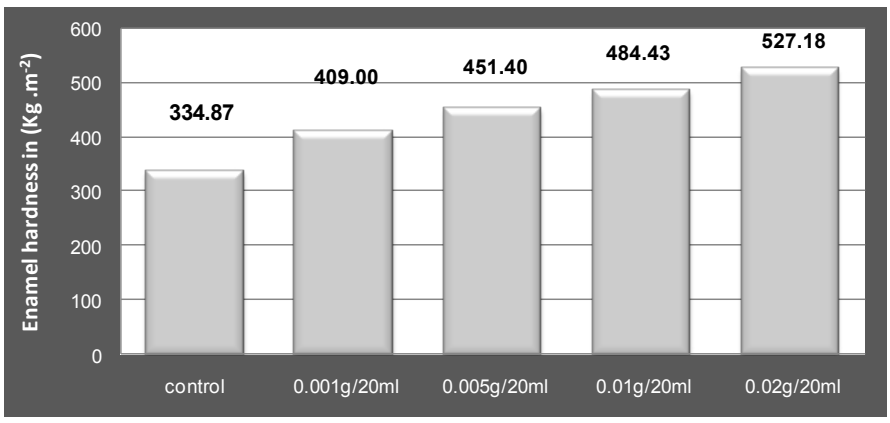
The results of the hardness are reported in Table (1) and figure (2) from which it is observed that the highest mean value obtained was in concentration 0.02g/20ml which had a mean value of 527.18 ± 2.904 Kg .m-2 , while the lowest mean value was for control group which had a mean value of 334.87± 2.904 Kg .m-2. The results showed a significant increase in hardness between groups in concentration 0.001g/20ml , 0.005 g/20ml , 0.01 g/20ml and , 0.02 g/20ml compared with control group. Peter Atkin`s and Julio de paula (20)

it's well known that CNTs are thin cylinders of carbon atoms that are mechanically strong. At this time, the increase in hardness due to the increase in concentration of MWCNTs figure (4), the tremendous surface area of CNTs up to 200 m2.g-1 leads to formation of clusters due to Van Der Waals forces. Clustering and non-uniform dispersion of CNTs will lead to inhomogeneous property distribution in the structural component (21). The results of the hardness are reported in Table (1) and figure (3) from which it is observed that the highest mean value of polished eamel surface which had a mean value of 452.59 ± 1.84Kg .m-2 ,while the lowest mean value of unpolished enamel surface which had a mean value of 430.16 ± 1.84 Kg .m-2 . The results showed a significant increase in the hardness for polished samples compared with unpolished samples. Because the surface area for polished samples more than unpolished samples. Since the of the polished surface leads to increases the surface area of dental enamel attached to them MWCNTs material, which leads to increased the permeability of the materials to the inside enamel rods ,this leads to increased material inside dental enamel, gives an increase in the enamel hardness (22).

Figure (1): Micro-Vickers hardness testing machine (CV-400 DM).

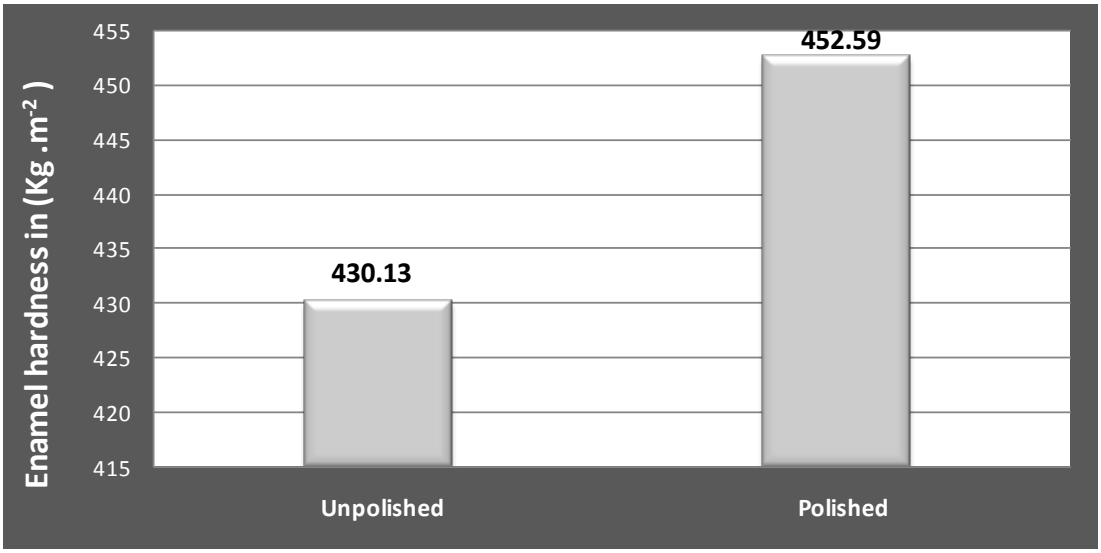
Table (1): Descriptive data of enamel hardness in (Kg .m-2) after MWCNTs application with DMSO of the relationship between different concentration treatment and different surfaces treatment

Concentration g.mol-1	Subjects no.	Unpolished	Polished	Mean ± S.E
Control	6	330.30 g	339.43 g	334.87 ± 2.904 e
0.001/20	6	396.27 g	421.73 e	409.00 ± 2.904 d
0.005/20	6	437.67 e	465.13 d	451.40 ± 2.904 c
0.01/20	6	479.27 cd	489.60 c	484.43 ± 2.904 b
0.02/20	6	507.30 b	547.07 a	527.18 ± 2.904 a
Mean		430.16 ± 1.84 b	452.59 ± 1.84 a	LSD0.01 (concentration) = 9.89
LSD 0.01 (surfaces) = 6.25				
Concentration vs surfaces LSD 0.01 = 16.02				



\s

Figure (2): Column chart illustrates the enamel hardness in (Kg .m^{-2}) after MWCNTs application with DMSO in different concentration treatment



\s

Figure (3): Column chart illustrates the enamel hardness in (Kg .m^{-2}) after MWCNTs application with DMSO in different surfaces treatment

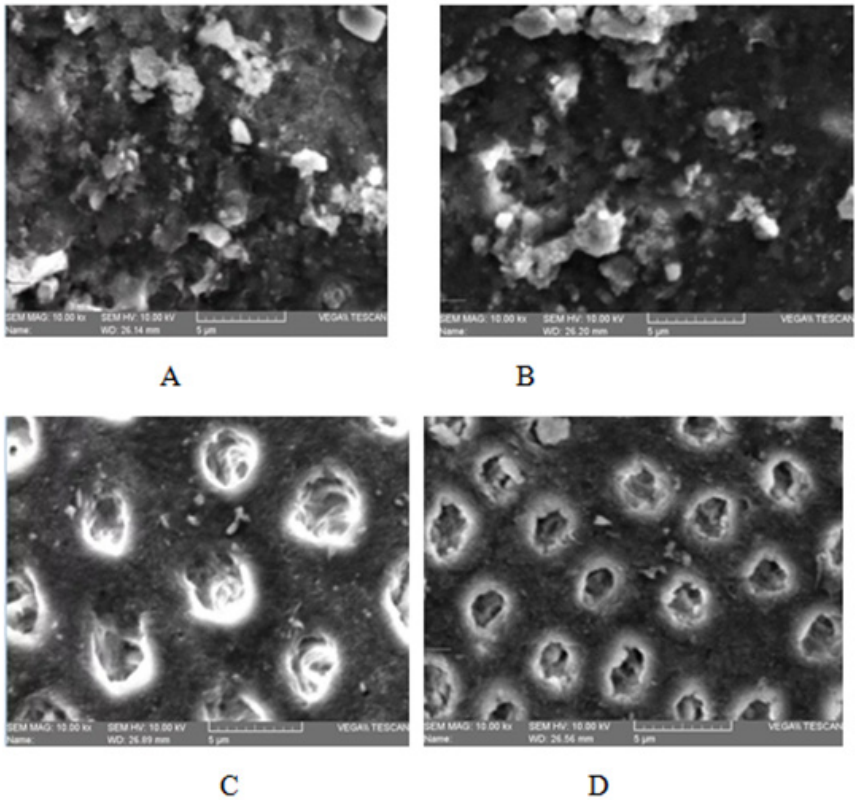


Figure (4):SEM: A:Unpolished sample without MWCNTs, B: Polished sample without MWCNTs, C: Unpolished sample with MWCNTs, D: Polished sample with MWCNTs in 5µm.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

Referances

1. Sabine B., Stefan H., Arndt K, Theo F. and Gerold A.S. The Fracture Behaviour of Dental Enamel. BIOMATERIALS.2011 ; 31: 375-384.
2. Marshall S.J., Balooch M., Breunig T., Kinney J.H., Tomsia A.P., Inai N., Watanabe L.G., Wu-Magidi I.C., Marshall G.W. Human dentin and the dentin–resin adhesive interface. ActaMater1998;46: 2529–2539.
3. Ten Cate A.R..Oral Histology: Development, Structure and Function, Mosby,St. Louis, MO.1994.
4. Robinson C., Connell S., Kirkham J., Shorea R. and A. Smith.. Dental enamel-abiological ceramic: regular substructures in enamel hydroxyapatite crystals revealed by atomic force microscopy. J. Mater. Chem. 2008;14: 2242-2248.
5. Habelitz .S., Marshall S.J., Marshall G.W. and Balooch, Jr.. Mechanical properties of human dental enamel on the nanometre scale. Arch. Oral. Biol. 2001;46: 173-183.
6. Cuy J.L., Mann A.B., Livi K.J., Teaford M.F. and Weihs T.P.. Nanoindentation mapping on the mechanical properties of human molar tooth enamel. Arch. Oral Biol. 2002;47: 281-291.
7. Kaehler T.. Nanotechnology: Basic concepts and definitions. Clin Chem 1994;40: 1797-1799.
8. Schleyer, T.L.. Nanodentistry. Fact or Fiction? J Am Dent Assoc 2000;131: 1567- 1568.
9. Lahn, J.. Nano's big future: Nanotech -National geographic. 2006.
10. Majumder, D.D.. IETE technical review. 2007;24:1: 9-25.
11. Hett, A.. Nanotechnology: Small matters, many unknowns, Swiss Reinsurance Company2004; 57;123-156.
12. Aitken R.J., Creely K.S., and Tran C.L.. Nanoparticles: An Occupational Hygiene Review,Institute of Occupational Medicine, Health and Safety Executive (HSE), UK, Research Report 2004;41:274, 113
13. Attin T., Meyer K., Hellwig E., Buchalla W. and Lennon, A.M.. Effect of mineral supplements to citric acid on enamel erosion. Archives of Oral Biology; 2003;48(11):753-759.
14. Chunmuang .S., Jitpukdeebodintra. S., Chuenarrom C. and Benjakul P. Effect of xylitol and fluoride on enamel erosion in vitro. Journal of Oral Science; 2007;49(4):293-297.
15. Ryge G., Foley D.E. and Fairhurst C.W. Microhardness and chemical composition of human tooth . J. Dent. Res. v. 1961;40: 1116-1121.
16. Meredith N., Sherriff M., Setchell D.J., Swanson S.A.V. Archs, Oral Biol. v. 1996;41: 539-545.
17. Salipira K.L., B.B. Mamba, R.W. Krause, T.J. Malefetse and S.H. Durbach. Cyclodextrin polyurethanes polymerised with carbon nanotubes for the removal of organic pollutants in water. Water SA., 2008;34: 113-118.
18. Panich M. and Poolthong S. The effect of casein phosphor peptideamorphous calcium phosphate and a cola soft drink on in vitro enamel hardness. J Am Dent Assoc; 2009;140: 455-460.
19. Potocnik I.; Kosec L. and Gaspersic D. Effect of 10% carbamide peroxide bleaching gel on enamel microhardness, microstructure, and mineral content. J Endod. 2000;26: 203-206.
20. Peter Atkin's and Julio de. P. Atkin's physical chemistry. 2006; 720-725.
21. Agarwal A., Bakshi S.R., Lahiri D. Carbon Nanotubes reinforced metal matrix composites. CRC Press Taylor and Francis Group, New York, ISBN: 2011;978-4398-1149-8.
22. Ajayan P.M. and Zhoy O.Z. Application of carbon Nanotubes in carbon Nanotubes, Topics Appl. Phys. 80, M.S. Dresselhaus , G. Dresselhaus, Ph. Avouris (Editors). 2001;391-425,Springer-Verlag.

Evaluation of CEA,CA19-9, and CA242 Tumor Markers in Patients with Colorectal Cancer by ELISA Technique

Ahlam Shakir Hammood AL- Rubaiawi[1] Mukhtar Khamis Haba[1], Laith Raouf Ahmed AL- Hadad[2]

[1]Department of Biology/ College of Science for Women/ University of Baghdad/Iraq., [2]Consultant Digestive Surgery/ GIT Hospital/ FRCS/ FACS/Iraq.

Abstract

Colorectal cancer (CRC) is one of the common malignant tumors of gastrointestinal tract (GIT). A total of 40 patients with CRC were studied preoperatively and postoperatively while only 20 healthy persons were studied. Their ages ranged between 18-78 years.This study was carried out to investigate the possible association of some risk factors with CRC such as (age, gender, smoking, inflammatory bowel disease, and ulcerative colitis).

With application of ELISA technique, Serum levels of CEA, CA 19-9, and CA 242 was measured preoperatively and postoperatively. The results showed an increase in preoperative serum level of CEA in CRC patients when compared to both postoperative and control groups, There was a significant increase in the serum level of CEA (10.73 ± 4.01) comparison with the two other groups (postoperative 3.95 ± 1.55) and control (1.91 ± 0.27) at ($P<0.05$), but no significant differences between the two groups (postoperative and healthy) which have mean (3.95 ± 1.55 and 1.91 ± 0.27) respectively. Also showed a significant increase in serum level of CA19-9 in patients preoperative (23.28 ± 1.86), who were differed significantly from the mean of serum in patients postoperative (18.63 ± 1.40) and control group (19.11 ± 1.46) at ($P<0.05$), When compared the serum level of CA242 revealed a significant increase in serum level of patients preoperative (19.58 ± 3.46) as compared to postoperative(8.75 ± 1.77) and control group(9.85 ± 2.22) at ($P<0.05$).

Key words: *Colorectal cancer, Biomarkers, Metastasis, CEA, CA19-9, CA242.*

Introduction

Cancer is a disease that occurs when control is lost on cell division and growth as well as on the metastasis of abnormal cells. Reasons for cancer are both intrinsic, i.e. infections, smoking, radiant sources, and extrinsic, i.e. genetic and metabolic mutations, along with abnormal immune responses and hormone levels [(1).]

Cancers start in the cells that line the inside of colon (the longest fragment of the large intestine) and rectum (occupies the last several inches prior to the anus) [(2).] Stool blood, altered movement of bowel, continuous tiredness, nausea, vomit, malaise, anorexia, abdominal distension and losing of weight are among the main symptoms of CRC [(3).] Ageing and disturbed life style were reported to be the major reasons for developing CRC, with genetic abnormalities affecting only minor cases [(4).] Dietary influences which contribute to higher are red meat which is over processed, alcohol consumption, IBD (inflammatory bowel disease) [(5).] Tumor develops under strong influences from genetic and epigenetic disorders, while the survival can be best prognosis based on immunological microenvironment [(6).]

Materials and Method

Blood samples (5 ml) were collected from patients with colorectal cancer (preoperative- postoperative) healthy persons (control) by using plastic syringes. Serum samples were prepared by centrifuging the blood (5000 rpm for 15 min).

Tissue Biopsies: Biopsies were collected from tissues of CRC patients, followed by fixation in buffered formalin (10 %), embedding in paraffin wax, and finally staining by using hematoxylin-eosin. Tissue samples were collected from histopathological laboratories of Gastroenterology and Liver Diseases Teaching Hospital, Baghdad.

Immunological Assessments:Enzyme Immunoassay for Determination of Serum Tumor Markers by Using ELISA Kit: Quantitative determination of serum levels of three tumor markers (CEA, CA19-9 and CA 242) in both patient and control groups was performed by means of ELISA (Enzyme Linked Immunosorbent Assay) .

Results

The study included 40 patients with colorectal cancer, 23 male (57.50%) and 17 female (42.50%), with an age range of 18-78 years .The selected patients were classified into two groups: the first group represented serum of preoperative patients and the second group was of serum of postoperative patients. These two groups were compared to the third healthy group which was used as control. The control subjects included 20 individuals, 11 males and 9 females with ages ranged between 24 and 58 years.

Distribution of patients with colorectal cancer according to different risk factors:

The age of the 40 adult CRC patients included in this study ranged between 18 and 78 years, the maximum numbers of colorectal cancer patients was found within age group more than 60 years (20: 50%), then 12 patients in the age group of 40-60 years (30%), and the minimum number was 8 patients in the age group of less than 40 years (20%). Results in Table (1) demonstrate that the total number of patients was 40, of which 23 were male (57.50%) and 17 were female patients (42.50%). This implies that colorectal cancer is more common in men than in women, with significant differences between the two genders at $P<0.05$, also the proportion of smoking included 21 smoker patients (52.50%) and 19 non- smoker patients (47.50%), with statistically non-significant difference.

Carcinoma groups grading of the present study revealed that well- differentiated adenocarcinoma (G1) was seen in 4 cases (10.00%) of the CRC group, while 30 cases (75.00%) of patients had moderate differentiated adenocarcinoma (G2), and 6 cases (15.00%) had poorly differentiated adenocarcinoma (G3).There were a significant differences at ($P<0.05$) among carcinoma groups according to their grading (Table 1).

In the current study, most of the patients had carcinoma as the type of colorectal cancer. Concerning the staging of the tumor, 6 patients (15.00%) had tumor that invaded the submucosa (T1), 12 (30.00%) had tumor that invaded the muscularis (T2), and 22 (55.00%) had tumor that invaded through the mascularis into the pericolorectal tissues (T3).

The results of our study showed that, among the 40 patients with CRC, 4 (10.00%) had genetic disease, 9 (23.00%) had polyp, 11 (27.00%) had ulcerative colitis, and 16 (40.00%) had diabetes mellitus type II. There were a significant differences at ($P<0.05$) among carcinoma groups according to the risk factors. Most of tumors are localized in different sites of colon. In this study, about 2 patients (5.00%) had tumor in the transverse colon, 3 (7.5%) in the right colon, 8 (20.00%) in the sigmoid colon, 12 (30.00%) in the rectum, and 15 (37.5%) in the rectosigmoid.

Serum Levels of CEA, CA19-9, and CA242 Tumor markers in patients with CRC According to their characteristics and risk factors:

Results in Table (32) exhibited a significant increase of serum levels CEA in patients with age range of 40-60 years as compared with those with age less than 40 years old. However, there were no significant differences between the serum levels of CEA in patients with age more than 60 years old and the other two groups at $P<0.05$.

Comparing serum levels of CA19-9 showed no significant effects of age in patients with age less than 40 years old, 40-60, and more than 60 years old.

Significant increases were recorded in serum levels of CA242 in patients with age range of 40-60 years as compared with serum levels in patients with age less than 40 years old and more than 60 years old at $P<0.05$. Also, the patients with age more than 60 had a significant increase in serum levels of CA242 in comparison with patients with age less than 40 years old at ($P<0.05$).

The results in table (2) show a significant increase in the serum level of CEA in females in comparison with that in males at $P<0.05$. Nevertheless, no significant differences were recorded in the serum levels of CA19-9 and CA242 between males and females. Also the results demonstrate a significant increase in serum level of CEA in smoker patients as compared with non- smoker patients at $P<0.05$. The statistical analysis showed non-significant difference in the serum levels of CA19-9 (and CA242 between smoker and non-smoker patients.

The results showed no significant difference in sera mean levels of CEA, CA19-9, and CA242 in patients with colorectal cancer in the T1, T2 and T3. while the relationship between serum levels of CEA and tumor grades of patients with colorectal cancer showed a significantly increased mean level of CEA in patients with G2 tumor as compared with G1 and G3 at P<0.05. There was no significant difference in mean levels of CEA between G1 and G3. The statistical analysis also showed non- significant difference in serum means levels of CA19-9 and CA242 in patients with G1, G2, and G3 (Table 2).

Table 1. Distribution of sample study of patients according to difference risk factors:

Factors		**Percentage (%)**	**P-value**
Age group (year)	Less than 40	8(20%)	0.0001 *
	40-60	12(30%)	
	More than 60	20(50%)	
Gender	Male	23 (57.50%)	0.0372 *
	Female	17 (42.50%)	
Smoking	Smoker	21(52.50%)	0.094 NS
	Non-Smoker	19 (47.50%)	
Grade of Tumor	Grade I	4 (10.00%)	0.0001 *
	Grade II	30 (75.00%)	
	Grade III	6 (15.00%)	
Stage of Tumor	Stage I	6(15.00%)	0.0001 *
	Stage II	12(30.00%)	
	Stage III	22(55.00%)	
Other diseases	Genetic disease	4 (10.00%)	0.0001 *
	Polyp	9 (23.00%)	
	Ulcerative colitis	11 (27.00%)	
	Diabetes mellitus type II	16(40.00%)	
Site of Tumor	Transverse colon	2 (5.00%)	0.0001 *
	Right colon	3(7.5%)	
	Sigmoid colon	8 (20.00%)	
	Rectum	12 (30.00%)	
	Rectosigmoid	15 (37.5%)	
* (P<0.05), NS: Non-Significant.			

Table 2: Serum Levels of CEA, CA19-9, and CA242 Tumor markers in patients with CRC According to their characteristics and risk factors:

Studied groups	Mean ± SE		
	CEA in serum(ng/ml)	CA 19-9 in serum(U/ml)	CA 242 in serum(U/ml)
Less than 40	2.88 ± 0.14 b	17.52 ± 2.04 a	4.16 ± 1.35 c
40-60	10.48 ± 5.67 a	25.09 ± 2.69 a	20.63 ± 4.64 a
More than 60	6.74 ± 2.43 ab	19.91 ± 1.37a	12.85 ± 2.34 b
LSD value	5.483 *	9.947 NS	6.808 *
P-value	0.0330	0.171	0.0457
Male	2.62 ± 0.34 b	21.68 ± 1.46 a	15.07 ± 2.47 a
Female	13.72 ± 4.92 a	19.98 ± 1.98 a	12.95 ± 3.43 a
LSD value	8.341 *	4.849 NS	8.194 NS
P-value	0.0098	0.488	0.608
Smoker	11.36 ± 4.04 a	20.94 ± 1.87 a	15.14 ± 3.04 a
Non-smoker	2.89 ± 0.43 b	20.97 ± 1.42 a	13.09 ± 2.66 a
LSD value	1.993 *	4.801 NS	8.112 NS
P-value	0.044	0.986	0.615
T1	2.56 ± 0.57 a	21.93 ± 2.72 a	16.70 ± 5.63 a
T2	10.92 ± 5.72 a	22.57 ± 2.74 a	17.67 ± 4.58 a
T3	6.69 ± 2.42 a	19.81 ± 1.38 a	11.57 ± 2.25 a
LSD value	8.993 NS	6.729 NS	11.370 NS
P-value	0.319	0.610	0.478
G1	3.53 ± 1.09 b	23.22 ± 2.84 a	17.20 ± 4.45 a
G2	9.50 ± 3.17 a	20.17 ± 1.49 a	12.46 ± 2.48 a
G3	3.03 ± 0.27 b	21.84 ± 2.69 a	18.30 ± 5.66 a
LSD value	5.993 *	7.288 NS	12.314 NS
P-value	0.0420	0.566	0.368

*(P<0.05), NS: Non-Significant
Means with different letters in same column differed significantly

Discussion

The development of colorectal cancer is caused by a combination of genetic and environmental factors. Epidemiology studies have revealed a number of risk factors for colorectal cancer including age, gender, smoking, family history of colon cancer or inflammatory bowel disease. Various individual characteristics or behaviors are included among the risk factors since they enhance the possibility to develop CRC. Age is one of the major risk factors for CRC. The risk of CRC increases with age, Several hypotheses have been suggested to find explanations for such a correlation between susceptibility to cancers and the progression in age, ageing of individuals is associated with increased and accumulated exposure to carcinogenic materials from the environment, In addition, the cellular mutation repair ability can be declined with increasing age [7].

Our study demonstrated that CRC is more common in men than in women. The incidence and mortality rates are higher by about 30 and 40%, respectively, in men as compared to women, while full explanation for this gender differences is not yet available, it is believed to be due to several factors such as higher exposure to carcinogens , differences in sex hormones, in addition to the interactions among the different factors [8].

Also the proportion of patients who smoked was higher than non-smokers; many studies have reported a higher risk of CRC among cigarette smokers, especially among those with a long history of smoking (9). Smoke from tobacco was reported to be one of the main carcinogen sources, such as heterocyclic amines, polycyclic hydrocarbons and nitrosamines. However, the evidence about the roles of these materials in development of colon cancer is insufficient (10).

Patients with adult onset type 2 diabetes show greater risk of CRC [11]. Despite that the two diseases have several risk factors in common, such as obesity and a sedentary lifestyle, the correlation between them remains even after accounting for physical activity, body mass index, and waist circumference [12]. Patients with chronic inflammatory bowel disease (IBD), who commonly suffer from inflammation in colon for extended periods, were reported to show a two-fold higher risk of CRC development [13]. The extent, severity and duration of ulcerative colitis (UC), the most frequently reported form of IBD, were shown to associate with an elevated risk to develop cancer , UC is a chronic IBD with an unknown reason, mainly influencing the large intestine mucosa [14]. Family risks of CRC are of a greater risk of developing the disease, with that history is mostly driven by genetic mutations and environmental factors [15]. Genetics contributes to CRC risk by both gene-regulated pathways directly involved in disease development and inherited mutations accounting for about 10% of cases [16]. The polyp is the classical model of colorectal cancer pathogenesis and it describes the phases of transition from benign tumors into colorectal cancer over many years, primary factors of polyp to CRC sequence are gene mutations, epigenetic alterations and local inflammatory changes [17].

Tumor markers:

Biochemical analyses are helpful to diagnose and to deal with cancer cases.,Various tumor markers have been studied with respect to gastrointestinal cancer, including (**CEA**, **CA 19-9**, and **CA 242**).

CEA (carcinoembryonic antigen):Is a cell surface glycoprotein that is produced in the GI tract and pancreas in the prenatal period and released to the blood, CEA belongs to a group of molecules known as carcinoembryonic protein [18]. Clinical studies indicate that the cancer antigen (CEA) is associated with the development of metastases in colorectal cancer, although the biological function of CEA is not fully understood [19]. Higher CEA concentrations can be also detected in a number of benign and non-neoplastic cases such as inflammations of the lung, liver, and colon.

Conclusions

1. In our study, we found that elderly age, male gender, smoking, and other infection, appeared to be the most possible association factors for colorectal cancer.

2. The appearance of tumor marker and their concentration are related to the genesis and growth of malignant tumors in patients, and it should correlate with tumor stages and grades.

3. There was a significant elevation in mean levels of CEA, CA19-9, and CA242 serum of CRC patients, when compared with healthy subjects.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they

have no conflict of interest.

Funding: Self-funding

References

1. American Cancer Society. Global Cancer facts and figures. Atlanta (GA), 2007; Inc. No. 861807 pp: 52.
2. Hamilton SR, Bosman FT, Boffetta P, et al. Carcinoma of the colon and rectum. In: WHO Classification of Tumors of the Digestive System. Bosman FT, Carneiro F, Hruban RH, Theise ND, eds. Lyon: IARC Press. 2010;134-46.
3. National Cancer Institute. February 27, 2014. Archived from the original on July 5, 2014. Retrieved June 29, 2014.
4. World Cancer Report 2014. World Health Organization. 2014. pp. Chapter 1.1.
5. Watson AJ, Collins PD. "Colon cancer: a civilization disorder". Digestive Diseases. 2011; 29 (2): 222–8.
6. Maby, P., Tougeron, D., Hamieh, M., Mlecnik, B., Kora, H., Bindea, G., et al. Correlation between densities of CD8C T-cell infiltrate in microsatellite unstable colorectal cancers and frame shift mutations: a rationale for personalized immunotherapy. Cancer Res. 2015; 75: 3446–3455.
7. Aaltonen L, Johns L, Järvinen H, Macklin J, Houlston R. Explaining the familial colorectal cancer risk associated with mismatch repair (MMR)-deficient and MMR-stable tumors. Clin Cancer Res. 2007; 13:356-61.
8. Lin JH, Zhang SM, Rexrode KM, et al. Association between sex hormones and colorectal cancer risk in men and women. Clin Gastroenterol Hepatol. 2013; 11: 419-424.
9. Terry P, Ekbom A, Lichtenstein P, Haile RW & Bond JH." Long –term tobacco smoking and CRC in a prospective cohort study".nt. J.Cancer.2001; 91(4):585-7.
10. Martinez ME & McPherson RS. "Cigarette smoking and alcohol consumption as risk factors for colorectal adenomatous polys". J. Natl. cancer. 1995; 87: 274.
11. Tsilidis KK, Kasimis JC, Lopez DS, Ntzani EE, Ioannidis JP. Type 2 diabetes and cancer: umbrella review of meta-analyses of observational studies. BMJ. 2015; 350: 7607.
12. Larsson SC, Giovannucci E, Wolk A. Diabetes and colorectal cancer incidence in the cohort of Swedish men. Diabetes Care. 2005; 28: 1805-1807.
13. Lutgens MW, van Oijen MG, van der Heijden GJ, Vleggaar FP, Siersema PD, Oldenburg B. Declining risk of colorectal cancer in inflammatory bowel disease: an updated meta-analysis of population-based cohort studies. Inflamm Bowel Dis. 2013; 19: 789-799.
14. Beaugerie L, Itzkowitz SH. Cancer's complicating inflammatory bowel disease. N Engl J Med. 2015; 372: 1441-1452.
15. Strum WB. Impact of a family history of colorectal cancer on age at diagnosis, anatomic location, and clinical characteristics of colorectal cancer. International Journal of Gastrointestinal Cancer. 2005; 2:121.
16. Johns, L.E.; Houlston, R.S. A systematic review and meta-analysis of familial colorectal cancer risk. Am. J. Gastroenterol. 2001; 96: 2992–3003.
17. Bernstein, C; Bernstein, H; Payne, CM; Dvorak, K; Garewal, H ."Field defects in progression to gastrointestinal tract cancers". Cancer Letters. 2008; 260:1–2.
18. Planner M. "Tumor markers". American cancer society. INC. 2002.
19. Gold P, Freedman SO. Demonstration of tumor-specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques. J Exp Med. 1965; 121: 439-462.

Knowledge about Emergency Contraception Pills among Primary Health Care Doctors in Baghdad\Al-Karkh Sector

Waleed Arif Tawfeeq[1], Amer Abaas[2], Zaid Abdulateef Abdulkareem[2]

[1]Family & Community Med Department/ College of Medicine/ Mustansiriyah University-Iraq,
[2]AlKakh health Center /Ministry of Health/Iraq

Abstract

Background: Unintended pregnancy is associated with an increased risk of problems especially when woman is not in her optimal health for childbearing. The problem of unintended pregnancy and its complication can be reduced by the use of emergency contraception.

Objective: To explore the knowledge of health care doctors in Baghdad/Al Karkh, Iraq regarding emergency contraception pills.

Participants and Method: A structured questionnaire was distributed to a total of 390 primary health care doctors (obstetrics and gynecology specialists, general practitioners and family physicians). The questionnaire contained two main domains: demographic characteristics and knowledge about EC.

Results: The majority of the sample were females 287 (73.6%), and the general practitioner (41.8%), family medicine specialists (34.6%). about one third of them were in age 40–49 years old, 72.1% of the physicians have no training about emergency contraceptive..

Conclusion: A deficit in knowledge shown by health care physicians lead to an insufficient use of emergency contraceptive pills methods.

***Key words**: Knowledge, Emergency Contraception, PHC physicians.*

Introduction

Emergency contraception (EC) refers to methods of contraception that can be used to prevent pregnancy after sexual intercourse. These are recommended for use within 5 days but are more effective the sooner they are used after the act of intercourse, EC pills prevent pregnancy by preventing or delaying ovulation and they do not induce an abortion. EC cannot interrupt an established pregnancy or harm a developing embryo[(1)]. Unintended pregnancy is associated with an increased risk of problems for the mom and baby. If a pregnancy is not planned before conception, a woman may not be in optimal health for childbearing[(2)]. The problem of unintended pregnancy and its complication can be reduced by the use of emergency contraception (EC) [(3)]. Unprotected intercourse that demand the use of emergency contraception include failure of barrier methods such as slippage, breakage or misuse of condom, sexual assaults, failed coitus interrupts, two or more consecutive missed oral contraceptive pills, or simply because intercourse was unexpected and therefore contraception had not been used[(4)].

Emergency Contraceptive pills can serve as a backup method and can reduce the number of unintended pregnancies and abortions. There are three types of ECPs: combined ECPs containing both estrogen and progestin, progestin-only ECPs, and ECPs containing an antiprogestin (either Mifepristone or Ulipristal Acetate). Progestin-only ECPs have now largely replaced the older combined ECPs because they are more effective and cause fewer side effects. Although this therapy is commonly known as the morning-after pill, the term is misleading; ECPs may be initiated sooner than the morning after-immediately after unprotected intercourse-or later-for at least 120 hours after unprotected intercourse. Combined ECPs contain the hormones estrogen and progestin. The hormones that have been studied extensively in clinical trials of ECPs and found to be highly effective and well

tolerated[5-7]. The antiprogestin mifepristone approved for use in many countries for early first trimester medication abortion and it is highly effective for use as emergency contraception, with few side effects (delayed menstruation following the administration of mifepristone is one notable side effect) [8].

The aim of the present study is to assess the knowledge about EC pills among healthcare physicians at Baghdad Al Karkh/Iraq

Participants and Method

A cross sectional survey was conducted among health care physicians in Baghdad Al Karkh District during the period from Nov 2017 to Mar 2018. The studied population included 390 physicians (Obstetricians & Gynecologists, General Practitioner, Family physicians) in primary health care (PHC) centers. They were recruited by convenient selection method. The questionnaires were delivered to participant personally by the researcher himself at their work place to ensure completing the questionnaire instantaneously. Anonymity of participants was insured.

Questionnaire: Structured questionnaire were developed as per objectives of the study, this questionnaire was adopted from study made in 2005 for Exploring Knowledge, Attitudes and Practices of EC in PHC provider Tribhuvan University with modification[9]. Each participant was asked to complete the questionnaire which consist of two elements; Sociodemographic information "gender, age, specialty, marital status, years of experiences and formal training" and EC knowledge in PHC "17 main questions and 6 sub questions".

Statistical Analysis

The data were coded and each questionnaire assigned with a serial identifying number then entered by the researcher into the computer using Statistical Package for Social Sciences (SPSS) version 24. Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values). The significance of difference of different percentages (qualitative data) were tested using Pearson Chi-square test with application of Yate's correction or Fisher Exact test whenever applicable. Statistical significance was considered whenever the P value was equal or less than 0.05.

Table 1: The distribution of PHC physician related to sociodemographic characteristics (n=390).

		No	%
Gender	Male	103	26.4
	Female	287	73.6
Specialty	General practitioner	163	41.8
	Family Medicine	135	34.6
	Obstetric & Gynecologists	36	9.2
	Others (Pediatrics, ENT, Rotator…etc.)	56	14.4
Age (years)	20---29	36	9.2
	30---39	107	27.4
	40---49	132	33.8
	50---59	95	24.4
	=>60	20	5.2
Marital status	Unmarried	70	17.9
	Married	320	82.1
Years of working experience (years)	0-9	108	27.7
	10-19	114	29.2
	=>20	168	43.1

Table 2: The distribution of PHC physician according to their knowledge about EC (n=390)

		No	%
Have you ever hear about EC		320	82.1
The source of information	College study	212	66.2
	Mass media	13	4.1
	Continuous medical education in PHCC	8	2.5
	MOH	77	24.1
	Others	10	3.1
Know the name of EC (n=320)		244	76.4
Method is: Same as combined oral contraception pills (COCs) and/or high dose of hormones (Ulipristal acetate (UPA))		283	88.4
Intra uterine device (Cupper T)		160	53.7
Have EC in the PHC center		84	26.2

Table 3: The distribution of PHC physician according to knowledge about mechanism of action, effectiveness, indication, safety, and testing for pregnancy before EC (n=320)

		No	%
Know mechanism of action		217	67.9
The mechanism of Action	Prevent implantation	55	25.3
	Prevent implantation & ovulation	68	31.3
	Induces abortion	81	37.4
	Do not know	13	6.0
Effectiveness of EC preventing pregnancy	Very good (>95%)	78	24.4
	Good (75-90%)	198	61.8
	Fair (50-74%)	17	5.4
	Don’t know	27	8.5
Safety profile of EC	Very safe	22	6.9
	Safe	249	77.9
	Cause health problems	35	10.8
	Not sure	14	4.4
Know the indication of EC		252	78.7
When contraception method has been used		144	57.3
Condom rupture		227	90.2
Condom used perfectly		143	57.0
Condom slippage		206	81.8
Correct coitus interruption		121	48.2

Cont... **Table 3: The distribution of PHC physician according to knowledge about mechanism of action, effectiveness, indication, safety, and testing for pregnancy before EC (n=320)**

Miscalculation of the periodic absent method		154	61.2
IUCD expulsions		168	66.8
When a woman had been a victim of sexual assault		204	81.1
Need to do pregnancy test before EC	Yes	101	31.5
	No	132	41.3
	Don't know	87	27.2

Results

Table 1 presents the demographic characteristics of the health care providers who completed the study questionnaire; the majority of them were females 287 (73.6%) while the rest were males (26.4%). Among them were general practitioner (41.8%), family medicine specialists (34.6%), obstetric & gynecological specialists (9.2%) and from other specialties (Pediatric, ENT, rotator … etc.) (14.4%), Regarding their age, about one third of them were in age 40–49 years old (33.8%), 27.4% were 30-39 years, 24.4% were 50-59 years, and 9.2% were 20– 29 years while about 5.2% were 60 years and above. Most of the PHC physician were married (82.1%) with working experience less than 10 years in 27.7% of them, 10–19 years (29.2%) and 20 years and above representing about half of them (43.1%).

The term EC was known by 82.1% of physician with their source of knowledge from college study (66.2%), MOH (24.1%), mass media (4.1%), continuous medical education in PHCC (2.5%) and others (brochures, reading, journals, internet e-mails….etc.) in 3.1% (Table 2).

Among 320 PHC physician who heard about EC 244 (76.4%) knew the trade name of the available types of EC. The majority of those 320 PHC physician mention that EC were same as COC pills and/or high dose of hormones "Ulipristal acetate" (UPA) (88.4%)

Table 3 reveals that 67.9% of PHC physician know the mechanism of action of EC as inducing abortion (37.4%), preventing implantation and ovulation (31.3%), only preventing implantation (25.3%), or no exact mechanism is known (6.0%).

Among 320 PHC physician, 41.3% of them think that the client no need to do pregnancy test before take EC and the rest either they trust their clients and consider need for pregnancy test before EC. More than half of the PHC physician (61.8%) consider the effectiveness of EC in preventing pregnancy is good, 24.4% as very good, while the rest either did not know (8.5%) or assume it as fair (5.4%). Regarding safety, more than two-thirds of physician (77.9%) believed that EC have a safe profile (Table 3).

Table 3 also demonstrates that larger number of PHC physician have known the right indication of EC (78.7%) as follow, condom rupture 90.2%, condom slippage 81.8%, for woman had been a victim of sexual assault 81.1%, IUCD expulsions 66.8%, miscalculation of the periodic absent method 61.2%, while those physician who mention the wrong indication was as follow, when contraception method has been used 57.3%, condom used perfectly 57.0%, correct coitus interruption 48.2%.

Discussion

Family planning programs in Iraq are unfortunately limited and based only on little consultancy clinics within PHCCs and the private clinics[10]. The use of contraceptive methods by Iraqi women reached a rate of 56.1%[11]. Present study showed that 82.1% of PHC physicians heard about emergency contraception (EC). This finding is lower than results of Batur *et al*[12] web based survey on 3260 practicing physicians in USA which revealed that 95% of physicians heard about levonorgestrel EC. However, rate of hearing about EC by our study is higher than that reported by Mandiracioglu *et al*[13] study in Turkey of 53.7% of PHC workers who heard about EC. Differences in hearing about EC proportions between studies might be attributed to discrepancy in quality of medical education and training of physicians between different countries. Current study revealed that 76.4% of PHC physicians know the name of EC. This EC knowledge rate is close to knowledge rate of family physician working in PHC reported by

Abdulghani *et al*[14] study in Pakistan of 71% and that of health care workers reported by Zeteroğlu *et al*[15] Study in Turkey of 74%.

However, knowledge rate of EC in present study is higher than results of Harrison cross sectional study in Nigeria which stated that only 45% of medical doctors could correctly defined the emergency contraception[16]. On other hand, knowledge of current study PHC physicians regarding EC is lower than that of 87% knowledge rate of physicians reported by Lo *et al*[17] study in Hong Kong. These differences in knowledge regarding EC between studies may be due to different reasons like differences in governmental interest in family planning programs, information given as part of the medical curriculum lectures in medical colleges or during postgraduate training and continuing medical education. Most of PHC physicians (88.4%) had knowledge in EC method of combined oral contraception pills (COCs) and/or high dose of hormones (Ulipristal acetate (UPA)). This finding is higher than results of Oriji and Omietimi study in Nigeria[18] which reported that although, 98% of medical doctors had good knowledge in EC, 58% of them could not recognize the EC types. More than half (63.8%) of PHC physicians believed that combined pills is the best choice. Current study showed that 67.9% of PHC physicians knew the emergency contraception mechanism of action and 37.4% of them thought that mechanism by induced abortion. This proportion of knowledge is higher than results of Ebuehi O *et al*study in Nigeria which found that 48.8% of health care providers have good knowledge in EC mechanism of action. Knowing mechanism of EC action is essential for physicians practice, effectiveness and dealing with complications[19].

This study showed that 24.4% of PHC physicians believed that EC is very good and 61.8% believed that it is good. These findings are close to Results of Lawrence *et al*[20] study in USA which revealed that 89% of physicians believed that EC and access to EC would lower the rate of intended pregnancy. Knowing emergency contraception indications was observed among 78.7% of PHC physicians in present study. The highest knowledge regarding the indications was for condom rupture (90.2%) and lowest for correct coitus interruption (48.2%). These findings are close to results of Fok study in USA which reported that 74.7% of physicians had proper knowledge regarding the indications of EC[21].

In conclusion, a clear deficiency in PHC physicians' knowledge about EC pills methods which leads to an insufficient use of EC pills methods.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. WHO. Emergency contraception. Available from: http://www. who.int/news-room/fact-sheets/detail/ emergency-contraception. Accessed on 23/7/2018.
2. `Mamboleo N. Unwanted pregnancy and induced abortion among female youths: a case study of Temeke district. Doctoral dissertation, Muhimbili University of Health and Allied Sciences, 2012. oai:ir.muhas.ac.tz:123456789/593. http://hdl. handle.net/ 123456789/593
3. Grimes DA, Benson J, Singh S, Romero M, Ganatra B, Okonofua FE, Shah IH. Unsafe abortion: the preventable pandemic. The lancet, 2006; 368(9550):1908-19.
4. Relwani N, Saoji A, Kasturwar NB, Nayse J, Junaid M, Dhatrak P. Emergency contraception: exploring the knowledge, attitude and practices of engineering college girls in Nagpur district of central India. Nat J Comm Med. 2012;3(1):14-9.
5. Fine P, Mathé H, Ginde S, Cullins V, Morfesis J, Gainer E. Ulipristal acetate taken 48–120 hours after intercourse for emergency contraception. Obstet & Gynecol. 2010; 115(2):257-63.
6. Glasier AF, Cameron ST, Fine PM, Logan SJ, Casale W, Van Horn J, et al. Ulipristal acetate versus levonorgestrel for emergency contraception: a randomised non-inferiority trial and meta-analysis. The Lancet. 2010; 375(9714):555-62.
7. Li HW, Lo SS, Ng EH, Ho PC. Efficacy of ulipristal acetate for emergency contraception and its effect on the subsequent bleeding pattern when administered before or after ovulation. Human Reproduction. 2016; 31(6):1200-7.
8. Shen J, Che Y, Showell E, Chen K, Cheng L. Interventions for emergency contraception.

Cochrane Database of Systematic Reviews. 2017(8).

9. Shrestha DL. Knowledge and attitude about emergency contraception among PHC providers at Dang district. 2002.
10. Iraq Reproductive Health Assessment Prepared by UNFPA/Baghdad, 2003. Available at: www.unfpa.org/rh/docs/iraq-rept04-08-03.doc
11. Ebrahim SM, Muhammed NK. Knowledge, attitude and practice of family planning among women in Basrah city south of Iraq. The Medical J of Basrah University 2011; 29 (1 & 2): 70-76.
12. Batur P, Cleland K, McNamara M, Wu J, Pickle S, EC Survey Group. Emergency Contraception: A multi-specialty survey of clinician knowledge and practices. Contraception, 2016; 93(2):145- 152.
13. Mandiracioglu A, Mevsim V, Turgul O. Health personnel perceptions about emergency contraception in primary health-care centers. Eur J Contracept Reprod Health Care 2003; 8(3):145-149.
14. Abdulghani HM, Karim SI, Irfan F. Emergency Contraception: Knowledge and Attitudes of Family Physicians of a Teaching Hospital, Karachi, Pakistan. J Health Popul Nutr, 2009; 27(3):339–44.
15. Zeteroğlu S, Sahin G, Sahin HA, Bolluk G. Knowledge and attitudes towards emergency contraception of health-care providers in a region with a high birth rate. Eur J Contracept Reprod Health Care 2004; 9(2):102-106.
16. Harrison AO. Knowledge and attitude towards emergency contraception among medical doctors in Delta State. Afr J Med Health Sci 2014; 13: 39-46.
17. Lo SS, Kok WM, Fan SY. Emergency contraception: knowledge, attitude and prescription practice among doctors in different specialties in Hong Kong. J Obstet Gynaecol Res 2009; 35(4):767-774.
18. Oriji VK, Omietimi JE. Knowledge, attitude, and practice of emergency contraception among medical doctors in Port Harcourt. Nigerian J of Clin Practice, 2011; 14(4): 428-31.
19. Ebuehi OM, Ebuehi OA, Inem V. Health care providers' knowledge of, attitudes toward and provision of emergency contraceptives in Lagos, Nigeria. Int Fam Plan Perspect 2006; 32:89-93.
20. Lawrence RE, Rasinski KA, Yoon JD & Curlin FA. Obstetrician- gynecologist physicians' beliefs about emergency contraception: A national survey. Contraception, 2010; 82(4): 324-30.
21. Fok WK. Physician Knowledge, Attitude, and Confidence with Emergency Contraception. Obstet & Gynecol, 2016; 127 (5): 115-16S.

Relationship of Vitamin D with Some Electrolytes in the Serum of People with Rheumatoid Arthritis in the City of Samarra

Adeeb Mahfooth Farag Al-Samaria[1], Kader Abdullah Shanak Al-Esawi[2], Esraa Ali Abdul Kareem Al-Samaria[3], Dhamer Ismael Madab AL-Khazraji[4]

[1]Iraq/Ministry of Education / Directorate General of Salah Uddin Education, [2]Iraq/ Ministry of Scientific Research-University of Fallujah , [3]Iraq/ Ministry of Scientific research-University of Samaria, [4]Iraq/Ministry of Education / Directorate General of Salah Uddin Education

Abstract

The study was conducted on 50 samples of 30 patients with rheumatoid arthritis. The samples were divided into 15 females, 15 males and 20 control group (healthy) without any disease. The sample was divided into 8 females and 12 males. 80 years) and collected samples from Samarra General Hospital and outpatient clinics of the city. The blood was then collected from the healthy and sick people and separated by centrifugation. Biochemical variables were measured (vitamin D, glutathione , Mallon dialdehyde , uric acid).

The results of the current study showed a significant decrease in the level of vitamin D in the serum of people with rheumatoid arthritis compared to healthy people, and the results showed a significant decrease in the level of glutathione in the serum of people with rheumatoid arthritis compared to healthy people, with no significant differences in Level of Mallon dialdehyde. We also note a significant increase in the level of uric acid in the serum of people with rheumatoid arthritis compared to healthy people.

Key Words :- *rheumatoid arthritis, Vit .D, Oxidative stress*

Introducation

Arthritis is often used to indicate any disorder affecting the joints(1), including symptoms such as Jointsstiffness, Joint pain, Redness, Swelling, difficulty in movement, and may affect other organs. Rheumatoid arthritis, rheumatoid arthritis, gout (2) osteoporosis usually occurs with age and affects the joints near the ends of the fingers at the base of the thumb and neck and below. Back, knees and hips. (3) Rheumatoid arthritis is an autoimmune disorder that often affects the shoulders, hands, knees and feet and also affects cartilage, tendons, and bones through swollen vertebral tissue (2).

Rheumatoid arthritis is a multifaceted form of disease whose causes, symptoms, and treatment are different (4). Rheumatoid arthritis is a common health problem affecting millions of people worldwide, leading to higher health care costs (5) Of the world's population, with a prevalence rate of 1-0.5% (6), while in Colombia it was 0.9% (7).

The treatment of arthritis is focused on alleviating the symptoms and improving the ability of the joints to function. It is sometimes necessary to experiment with various treatments or to combine different treatments with each other in order to be able to determine the best treatment for the patient. There are many drugs used to treat inflammation (8) Including analgesic drugs, nonsteroidal anti-inflammatory drugs or some dietary supplements such as Glucoseamine and Chondroitin sulfate. Studies have shown that corticostroids use longer and higher, leading to peripheral joints. (9) There are many environmental factors that increase the risk of rheumatoid arthritis and the most serious risk is smoking. Previous studies have shown that smoking increases the development of the disease, especially in patients who have a positive result of ACP (10) Environmental factors are not just smoking, but factors such as food, alcohol, vitamin deficiencies, viruses and bacteria. (11) These factors are all outside the body and have no genetic basis, so they are called non-genetic factors (12) Epstein Barr Virus (EBV) was also associated with rheumatoid arthritis. There was an abnormal increase in the number of B lymphocytes infected with this virus in the blood of the rheumatoid arthritis patient. This virus stimulates the

production of antibodies Including the rheumatic factor [13].

Vitamin D is a type of fat soluble vitamin, and sun exposure is the main source for the body's needs of this vitamin, so it is called vitamin sun rays, which is different from the rest of the vitamins not necessary from food sources for this reason enough exposure to radiation Sun for 10 to 15 minutes a day on sunny days, and two to three times a week to get vitamin D requirements in the majority of people [14].

Vitamin D is of biological importance to the body of the organism. It is essential for balance of calcium, bone growth and regulation of the immune system. Its deficiency can cause Rickets disease, osteoporosis, osteoporosis and muscle weakness. Vitamin D deficiency is linked to cancer, cardiovascular disease and schizophrenia. Arthritis, type 1 diabetes, IDDM, psoriasis and vitiligo [15], as well as a decrease in risk associated with increased mortality [16] and increased risk of breast cancer [17].

Oxidative stress is defined as the imbalance between free radicals (ROS), Reactive Nitrogen Species (RNS), and antioxidants, which are important indicators of many pathological conditions, including atherosclerosis [18], as well as disorders Heart, blood vessels and arthritis [19] and an imbalance between it and antioxidants may also cause an increase in blood pressure Hypertension [20].

Since vitamin D may be related to rheumatoid arthritis, the current research target for measuring vitamin D in patients with rheumatoid arthritis.

Material and Method

Collection of specimens

The study was conducted on 50 samples of 30 patients with rheumatoid arthritis. The samples were divided into (15 females, 15 males) and 20 control groups (healthy) without any disease. The sample was divided into 8 females and 12 males. 80 years) and collected samples from Samarra General Hospital and outpatient clinics.

Blood collection

Collect about 6 cm 3 of the blood of healthy and sick people and is divided according to the type of test. The blood is placed in the Jell tubes. It has an airtight cover, free of anticoagulant, leaving the blood at 25 ° C until it coagulates and then placed in the centrifuge for 10 minutes at 3000 cycles / Minute and then the serum was obtained and then placed in small test tubes and kept in the refrigerator at a temperature of 20 - M for the purpose of measuring the biochemical variables, including (vitamin D, glutathione , Mallon dialdehyde, uric acid).

Estimation of Vitamin D

The level of vitamin in the serum was assessed using the Kit kit and manufactured by German company Human by method (21).

Estimation of antioxidant

Glutathione-GSH was estimated by Sedlak, Tietz (22,23). The concentration of uric acid in the serum was estimated using the enzymatic method using several prepared analyzes prepared by the Tunisian Biomegrheb (24).

Estimation of Oxidative stress

Determination of the level of Mallon dialdehyde -MDA in Guidet (25).

Statistical analysis

The results of all tests were analyzed using statistical program SPSS14, mean Mean and standard deviation (SD), and the special differences between the infected groups and the control group were determined using T.Test [26]

Result and Dissection

Measuring the level of vitamin D

The results showed a significant decrease in serum vitamin D levels in people with rheumatoid arthritis compared to healthy subjects as shown in Figure 1.

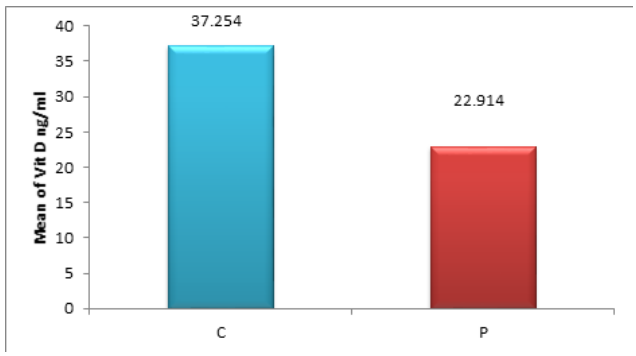


Figure (1): - Vitamin D level in serum samples of study and control groups

Vitamin D deficiency may lead to rheumatoid arthritis. Lee (2016) [27] points out that vitamin D decreases in people with rheumatoid arthritis, which is consistent with current research results. Azzen (2012) [28] found that vitamin D has a role in reducing the risk of rheumatoid arthritis, as there are many immunosuppressive effects including vitamin D as there is a potential relationship between vitamin D deficiency and autoimmune disease [29].

Vitamin D has been studied as an important and potential measure of the causes of many diseases, including rheumatoid arthritis. [30] The reason for vitamin D deficiency may be due to insufficient absorption of the vitamin D, which may be associated with exposure to sunlight [31]. Athanassion (2012) [32] also noted that vitamin D deficiency is very widespread in patients with rheumatoid arthritis and that its deficiency greatly increases the disease. There should also be vitamin D supplementation to prevent osteoporosis as well as relieve pain for patients with rheumatoid arthritis. [33] (2017), Hamad, noted that vitamin D levels were lower in rheumatoid arthritis patients as there should be comprehensive research studies on the role of vitamin D in the development of rheumatoid arthritis and its relationship to disease activity. Vitamin D has the function or function of regulating immunity associated with the potential effectiveness of vitamin D receptors. These include the treatment of many diseases, including rheumatoid arthritis and psoriasis, as well as many diseases [34].

Glutathione concentration measurement

Result shows the mean ± morbidity of the GSH level as the results showed a significant decrease in the level of GSH in the serum of people with rheumatoid arthritis in healthy people as in Figure (2).

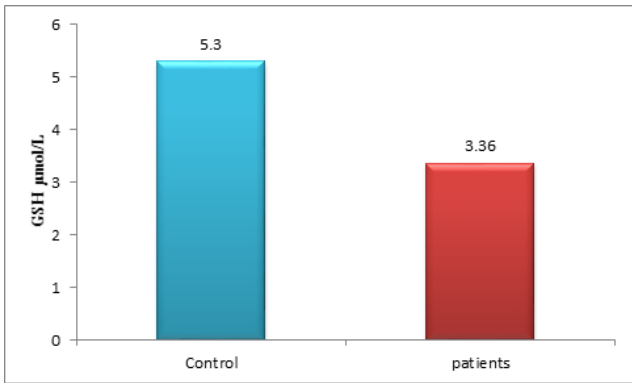


Figure (2): - Glutathione level in serum samples of study and control groups

In its study, Al-Maamory (35) reported a significant decrease in the level of glutathione in the serum of people with rheumatoid arthritis compared to healthy subjects, which is consistent with the results under study.

The low level of glutathione is due to several reasons, including the increase in the rate of consumption, which is one of the most important non-enzymatic antioxidants in the removal of free radicals and their products, transforming from the effective form to the ineffective form. The sulfur group in the GSH structure is a low-efficient factor. Between sulfur and hydrogen (SH) and the power of kin between carbon and hydrogen (CH) in the free radicals so they protect the cellular membranes from damage to free radicals.

One of the reasons for the low level of GSH is a deficiency in the raw materials of its structure, in particular the adjuvant enzyme (the reduced form) nicotine amide adenine deoxyribonide phosphatase, which is the catalyst for the action of the enzyme GRd, which works to restore the effective form of the collation of the ineffective form (36).

Measurement of Mallon dialdehyde

Result shows the mean ± standard deviation of the level of Mallon dialdehyde. The results showed that there was no significant difference in the concentration of Mallon dialdehyde in serum group of infected and healthy people as in Figure (3).

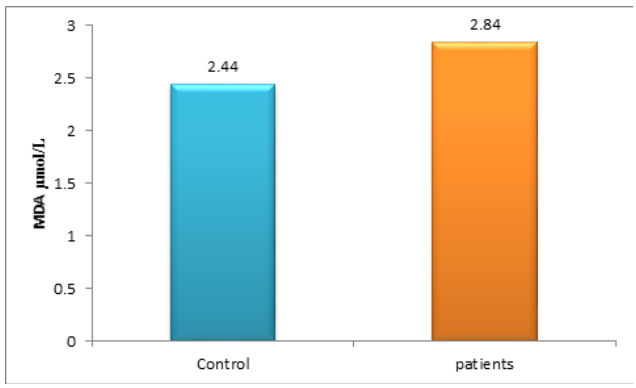


Figure (3): - Concentration of Mallon dialdehyde in serum samples of study and control groups

The results are not consistent with both Abbas (2011) (37) and 2008 (AL-Maamory)(35), who noted in their study that there was a significant increase in the level of MDA in the serum of people with rheumatoid arthritis.

Measurement of uric acid

Result shows the average ± standard deviation of uric acid level. The results showed a significant increase in serum uric acid concentration in people with rheumatoid arthritis compared to healthy subjects as shown in Fig. 4.

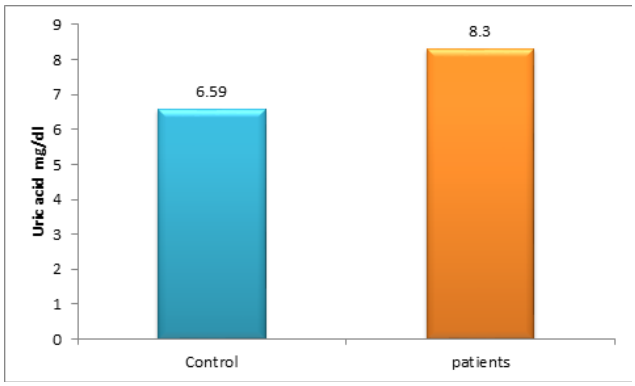


Figure (4): - Concentration of uric acid in the serum samples of the study and control groups

Sheania (2011) reported a significant increase in the level of uric acid in the serum of rheumatoid arthritis patients and this is consistent with the results under study. Das (2014) [39] indicated a rise in serum uric acid levels in people with rheumatoid arthritis.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Michel J B ,Shen Y K, Aiden A P , VeresA, GrayM K, *et al*., . Quantitative analysis of culture using millions of digitized books. Science, 2011;331(6014): 176-182.
2. SanthoshT R, Moin A , Gowda D V, Abhilasha T P , R Lakshmeesha N P, Patil R A. M. and Osmani. Intra-articular drug delivery system for arthritic diseases: overcoming the inadequacies of therapy. Indo. Amer. J. Pharmaceut. Res., 2016; 6:4427-4435.
3. Berenbaum F, Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!). Osteoarthritis and Cartilage.2013; 21(1): 16-21.
4. Erin T , Gregory D J , Khaled A E, and Braden C F , Animal Models of Osteoarthritis: Challenges of Model Selection and AnalysisThe AAPS Journal . 2013;15 (2) : 438-446 .
5. Carvalho AN., FiruziO , Gama MJ, Horssen Jvan, , Saso L. Oxidative stress and antioxidants in neurological diseases: is there still hope? Current drug targets. 2014.
6. Riemsma R P ., Taal E., Kirwan J R, and Rasker J J, Systematic review of rheumatoid arthritis patients education. Arthritis Care Res., 2004; 51(6): 1045-1059.
7. Díaz-Rojas J A, A.Dávila-Ramírez F, Quintana-López G, Aristizábal-Gutiérrez, F. and Brown P, Rheumatoid arthritis prevalence in Colombia: An approach based on burden of disease study during 2005. Revista Colombiana de Reumatología , 2016; 23(1):11-16.
8. Williams HJ, Ward JR, Egger MJ, et al .Comparison of naproxen and acetaminophen in a two-year study of treatment of osteoarthritis of the knee. Arthritis Rheum. 1993; 36:1196-1206.
9. Yurnbe T . Sami M, K Nishida. et al . Accelerated development of cervical spine instabillties in rheumatoid arthritis : a prospec live minimum s.year cohort study PLOS ONE a (2) Article ID e88970. 2014.
10. Liao KP, L .Alfredsson, E.W. and Karlson,. Environmental infection on risk for rheumatoid arthritis Curr Opin Rheumatol., 2009;21(3): 279-283.
11. Tobón G J T, Pierre Y, and Alain S. The environment, geo-epidemiology, and autoimmune disease: Rheumatoid arthritis. Autoimmun Rev., 2009; 9(5):1-5.
12. SilmanA J , Pearson J E. Epidemiology and genetics of rheumatoid arthritis. Arthritis Res. 2002; 4 (3): S2-65.
13. Balandraud N, and Roudier J. Epstein-Barr virus and rheumatoid arthritis. Rev. Rhum., 2017. 1-17.
14. Kathleen M.L, and Sylvia ES. Krause's Nutrition & Diet Therapy, The United States of America: Saunders, 2004 ;83-88.
15. Srirama L , Serum concentration of 25-hydroxy vitamin D in psoriatic patients in a tertiary care hospital – A case control study . IAIM 2016 ; 3 (1) : 97-107 .

16. Pilz S, Gruebler M, Gaksch M, et al . Vitamin D and mortality . Anticancer research , 2016 ; 36 (3) : 1379-1387 .

17. Elizabeth T J, Lindsay N K, and Peter W J, VITAMIN D and Colorectal , Breast , and , prostate Cancers : A Review of the Epidemiological Evidence , JOURNAL OF CANCER . 2016; 7(3): 232–240.

18. Rosensonm RS, and Stafforini DM. Modulation of oxidative stress, -associated phospholipase inflammation, and atherosclerosis by lipoprotein A2. J. Lipid Res. 2012; 53(9): 1767-1782.

19. Carvalho AN, Firuzi O, Gama M J, van Horssen J, L Saso. Oxidative stress and antioxidants in neurological diseases: is there still hope? Current drug targets. 2014.

20. Mohiuddin YH, Jawad AHl, College of Science for Girls. Baghdad Journal of Science. 13. 2016.

21. Heaney RP. Deficieacy of vitamin D, Clinical Laboratory International . 2010; 34,16 -19

22. N. W. Tietz .Textbook of clinical chemistry. 3rded. C.A.Burtis, E.R.Ashwood, W.B.Saunders. 1999; 819-861,1245-50

23. Sedlak J. and Lindsay R H .Analytical biochemistry. 1968; 192.(Cited by Al-Zamyle2001).

24. Kind P R, and, King E J. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J. Clin. Path.;7:322-326. 1954.

25. Guidet B, and shah S V, Am J. Physiol.1989; 257(26):440. (cited by Muslih, R. K.; Al-Nimer,M. S and Al-Zamely,O. Y., The level of Malondialdehyde after activation with H2O2 and CuSO4 and inhibition by deferoxamine and Molsidomine in the serum of patient with acute Myocardial infarction. National. J. chem. 2002; 5: 139-48.

26. Al-rawiea KM. Introduction to Statistics. Second Edition. Faculty of Agriculture and Forestry Mosul. 2000.

27. Lee.Basesc YH. Vitamin D level in rheumatoid arthritis and its correlation with the disease activity : a meta-analysis . Clin Exp. Rheumatal.2016;34(5)827-833.

28. Azzen FS. Relationship between vitamin D and Rheumatiod Arthritis Disease. Pakistan Journal of Nutrition .11(3); 2012: 293-300.

29. Szodoray p, N. B, GAAL J et al.: The complex role of vitamin D in autoimmune diseases. Scand J Immunol 2008; 68: 261-9.

30. GATENBY. P, LUCAS R, SWAMINATHAN A: Vitamin D deficiency and risk for rheumatic diseases: an update. Cur Opin Rheumatol2013; 25: 184-91..

31. Larry E, Johnson .Vitamin D: The Merck Manual of Diagnosis and Therapy . 2007; 33 :92- 98.

32. Athanassion I k, Athanassion P. A. Lyraki. et al. Vitamin D and rheumatoid arthritis. Therapeutic Advances in Endocrinology and Metabolism .2012. 3(6): 181-187.

33. Hamad B.A , Media S.K. Relationship between Vitamin D3 level and rheumatoid arthritis patients attending Rizgary Teaching Hospital in Erbil city . Zanco.J.Med.Sci, 2017. 21(2):

34. Nagpal S N, Singing and,R. Radhakrishnan Noncalcemic actions of Vitamin D receptor ligands .Endocrine reviews. 2005; 26(2005): 662-687.

35. Al-Maamory Fatma Abbass Dawood .Oxidative strees and serum lipid profile in Rheumatoid Arthritis .University of kufa.Collage.of Medicine .Dgree of Master. 2008.

36. Fisher, C J. Lipid hydroperoxide (LOOH) of the fatty ‡acid Organoselenium compounds as ‡Glutathione peroxidase mimics'' . B – 180 medical Laboratories ‡free radical and radiation biology program, the university of ‡Iowa; 2003;77: 222.‡

37. Abaas, H.F. Study the effect of body vitamins in the blood of rheumatoid arthritis, J of Sci. of Mus.5(12).2011

38. Sheanair D, Adul.Mounthe,Mohammed,Khasim M.M. et alThe realionship between serum limitations in Rheumatoid arthritis from Qurna districl,Basrah city .J.Kerbula.Un.2011 9(2).

39. D. Das, Bhatachary I., Saxena R.Saxena R., Lal.A.M. Relationship between uric acid and Ascrobic acid in Rheumatoid Arthritis patients. Diganta Das et al., sch App.Med.Sci: 2014;2:1711-1714.

Study of Thyroid Hormones for Vitiligo Patients in AL-Anbar Governorate

Yasser Q. Hussein[1] Wajeeh Y. Mohammed[1] Abdulla S. Al-Hasan[2], Sabri M. Al-marsomi[1]

[1]Iraq/University of Anbar – College of ScienceBiology Dep.,
[2]Iraq/University of Anbar – College of Medicine/Microbiology Dep.

Abstract

Vitiligo is a common acquired depigmentation disorder characterized by the loss of functional skin and mucosal melanocytes the reported incidence is 0.5% to 2% worldwide. Its pathogenesis is mostly consider autoimmune and this pigmentary disorder is strongly associated with autoimmune thyroid disorders. To evaluate the serum thyroid hormone in vitiligo patients of Al-Anbar governorate and to compare the results with other external studies. The study group included 80 patients with vitiligo, and 40 healthy volunteers. Blood thyroid hormone was determined using Monobind kits from reliable USA company. Their ages ranged form 1 to 70 years. Family history of vitiligo was positive in a percentage of (30%) of the patients. The mean levels of serum thyroid hormone (T3, T4) in patients with vitiligo were found to be no significant difference than those of healthy individuals. The TSH level in patient with vitiligo were found to be significantly higher than those of healthy individuals. This study strengthens the relationship between the thyroid hormones intake, formation, and metabolism with the pathogenesis of vitiligo. Therefore it is concluded that vitiligo patients should be evaluated for thyroid hormones.

Key Words: *Vitiligo, Thyroid hormone, Autoimmune thyroid,T3, T4, TSH.*

Introduction

Vitiligo is an acquired hypopigmentary skin disorder affecting the population of worlds without discrimination of race, age, gender and ethnic background(1). It is characterized by the formation of white patches and these patches associated with the loss of local melanocytes. Vitiligo involves the progressive loss of epidermal melanocytes and sometimes hair follicle melanocyte(2). Vitiligo is more significant in population of dark skinned individuals, due to its pigmentary disfigurement and has major impact on quality of life of patients(3). It produces social stigma in the affected individuals and is often confused with leprosy or other socially terrifying infectious diseases. It is a non-contagious disorder and the most commonly acqurid hypomelanosis(4). The lesions in vitiligo patients are most commonly found on the body areas that are exposed to sun like hands, arms, faces, feet etc. . Patients suffering from vitiligo may have premature graying of the scalp hair and eyebrows along with the appearance of white patches on the skin. Vitiligo is linked with simultaneous occurrence of other autoimmune diseases as well as psychosocial difficulties (5)(6)(7). In certain cultures, patients having vitiligo are regarded as social outcasts(3). Several reports have suggested associations between vitiligo and a variety of other autoimmune diseases, including thyroid conditions, alopecia areata, type 1 diabetes mellitus, pernicious anemia, and rheumatoid arthritis. Autoimmune thyroid diseases are common in patients with vitiligo(8). Studies have reported that the incidence of thyroid disease is 0-52% in patients with vitiligo, and that 3% to 90% of vitiligo patients have antithyroid antibodies(9)(10). Therefore, routine screening for thyroid dysfunction is recommended for patients with vitiligo(11).

Patients and Method

A total 80 patient with vitiligo were enrolled. Half number of healthy individuals with matching ages were included as controls. The samples were collected from the patients during their visiting to dermatological clinic of Dr. Abdullah salih Alhasan in Al-Anbar governorate. The ages of the patients ranging between 1-70 years old from both sexes. Many questions were asked to the patients about his name, age, accommodation, occupation, chronic diseases, family history, time of

infection, the presence of psychological disturbances, smoking, most common diet, most common drinks, spiritual questions (prayer), time of disease exacerbation, and the factors that exacerbate vitiligo to avoid the interferences with the other diseases, and to find a cause for this disease. All of patients and healthy individuals were not smokers, have no any chronic diseases, and not alcoholic drinkers. A total of (10 ml) of venous blood was drawn in sterile syringe and centrifuged to separate the serum and then stored at -45□C until begin used. The estimation of triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH) levels were done by using a Microplate Enzyme Immunoassay(12)(13)(14) from Monobind company (made in USA).

Results

The study included a total of 120 persons. Among them 80 had vitiligo (30 male and 50 female) and 40 were healthy controls (8 male and 32 female). Their ages ranged from 1 to 70 years . Family history of vitiligo was positive in a percentage of (30%) of the patients. The duration of disease ranged between 1 month to 20 years. History of seasonal variation of disease was positive in (60%) patients. Out of these (10%) noticed exacerbation of disease in winter while (50%) in summer season. Bad emotional state exacerbates of about (70%) of vitiligo patients, while the other (30%) dose not affected. This study showed that there is no relationship between the occupation, accommodation, most common diet, most common drinks, and spiritual side and vitiligo. In the patient's group T3 and T4 were not significant statistically than those in control group. while TSH was significantly higher than those in control group. The results are depicted in Table 1. The values are reported as mean ± SD and 95% confidence interval. For statistical analysis between groups paired t test was used. Pearson test was used for correlation analysis. The levels of each marker were compared between the study groups and control group, using SPSS computer package. P values of ≤ 0.05 were considered significant. The table above shows that T3 and T4 are not significant difference between patients and controls($P \geq 0.05$). While TSH is significantly higher in patients in a comparison with controls ($P \leq 0.05$).

Table 1: the mean value ,S.D, t-value, and p-value of the parameters were tested.

No.	parameters	factor	Mean ± SD	t- value	P-value
1	T3 ng/ml	patient	1.03±0.30	-0.429	0.669
		control	1.05±0.17		
2	T4 µg/dl	patient	7.69±1.86	0.000	1.000
		control	7.69±1.55		
3	TSH µIU/ml	patient	3.33±2.27	2.920	0.004
		control	2.23±0.96		

Discussion: Thyroid functional disorders and autoimmune thyroid diseases have been reported in association with vitiligo, and it seems that the incidence of clinical and subclinical thyroid involvement the incidence of clinical and subclinical thyroid involvement is more common in vitiligo patients than controls(15)(16)(17). Many researches about vitiligo and thyroid disease have been done with different results, one of these Mubki et al.,(2017)(18) showed that thyroid functional abnormalities were generally found more in vitiligo patients were approximately 1.6 times more likely to have abnormal TSH than control. The mean TSH level was overall higher in the vitiligo group. Both high TSH and low TSH levels were seen more frequently in vitiligo patients. The vitiligo group had significantly higher prevalence (5%) of primary hypothyroidism (high TSH and low T4) as compared to the control group. Alissa et al.,(2011)(19) and Akay et al.,(2010)(20) showed the predominance of females among vitiligo patients can be attributed to the fact that females are more conscious

about their cosmetic appearance and thus more likely to seek medical attention. Vitiligo seems to be commonly associated with autoimmune diseases . Two studies have reported associated autoimmune disease in (19%) and (23%) of vitiligo patients(21)(22).

One of the most commonly reported associations is thyroid disease, especially Hashimoto's thyroiditis(23). The reported prevalence of thyroid disease in the liteure ranged from (4%) to (21%) to even higher in other studies(22)(24)(25).

Conclusions

From our results we conclude that a strong relationship is found between the thyroid hormones and the pathogenesis of vitiligo.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Zhang Y, Mooneyan-Ramchurn JS, Zuo N, Feng Y, Xiao S. Vitiligo nonsurgical treatment: A review of latest. Dermatol Ther. 2014; 27(5): 296-303.
2. Bilal A, Anwar I. Guidelines for the management of Vitiligo. J Pak Assoc Derma.2014; 24(1): 68-78.
3. Parsad D, Dogra S, Kanwar AJ. Quality of life in patients with vitiligo. Health Qual Life Outcomes. 2003; 1: 58.
4. Taieb A. Intrinsic and extrinsic pathomechanisms in vitiligo. Pigment Cell Res. 2000; 13(8): 41-47.
5. Kostopoulou P, Jouary T, Quintard B, Ezzedine K, Marques S, Boutchnei S, Taieb A. Objective vs. subjective factors in the psychological impact of vitiligo: The experience from a French referral centre. Br J Dermatol. 2009; 161(1): 128-133.
6. Mashayekhi V, Javidi Z, Kiafar B, Manteghi AA, Saadatian V, Esmaeili HA, Hosseinalizadeh S. Quality of life in patients with vitiligo: A descriptive study on 83 patients attending a PUVA therapy unit in Imam Reza Hospital, Mashad. Indian J Dermatol Venerol Leprol.2010; 76(5): 592.
7. Wang KY, Wang KH, Zhang ZP. Health-related quality of life and marital quality of vitiligo patients in China. J Eur Acad Dermatol Venerol. 2011; 25(4): 429-435.
8. Iannella, G., Greco, A., Didona, D., Didona, B., Granata, G., Manno, A., ... & Magliulo, G. Vitiligo: pathogenesis, clinical variants and treatment approaches. Autoimmunity Reviews. 2016;15(4): 335-343.
9. Al-Mutairi, N., Kumar Sharma, A., Al-Sheltawy, M., & Nour-Eldin, O. Childhood vitiligo: a prospective hospital-based study. Australasian journal of dermatology. 2005; 46(3): 150-153.
10. Zettinig, G., Tanew, A., Fischer, G., Mayr, W., Dudczak, R., & Weissel, M. Autoimmune diseases in vitiligo: do anti-nuclear antibodies decrease thyroid volume?. Clinical & Experimental Immunology.2003; 131(2): 347-354.
11. Bae, J. M., Lee, J. H., Yun, J. S., Han, B., & Han, T. Y.Vitiligo and overt thyroid diseases: a nationwide population-based study in Korea. Journal of the American Academy of Dermatology.2017; 76(5): 871-878.
12. Gharib H., Ryan R. Mayberry W. et al. Radioimmunoassay for Triiodothyronine (T3): Affinity and Specificity of Antibody for T3 , J Clinical Endocrinol.1971; 33,509.
13. Barker S. Determination of Protein Bound Iodine , Journal Biological Chemistry.1984; 173, 175.
14. Hopton M, and Harrap J.Immunoradiometric assay of thyrotropin as a first line thyroid function test in the routine laboratory, Clinical Chemistry.1986; 32, 691.
15. Kasumagic-Halilovic, E., Prohic, A., Begovic, B., & Ovcina-Kurtovic, N. Association between vitiligo and thyroid autoimmunity. Journal of thyroid research. 2011.
16. Hegedüs, L., Heidenheim, M., Gervil, M., Hjalgrim, H., & Høier-Madsen, M. High frequency of thyroid dysfunction in patients with vitiligo. Acta dermato-venereologica.1994; 74(2): 120-123.
17. Niepomniszcze, H., & Amad, R. H.Skin disorders and thyroid diseases. Journal of endocrinological investigation.2001; 24(8): 628-638.
18. Mubki, T., Alissa, A., Mulekar, S., Albargawi, S., Youssef, M., & AlJasser, M.Association of vitiligo with anemia, vitamin B12 deficiency,

diabetes mellitus, and thyroid dysfunction in Saudi Arab patients: A case control study. Journal of Dermatology & Dermatologic Surgery.2017; 21(2): 72-76.

19. Alissa, A., Al, A. E., Huma, R., & Mulekar, S.Vitiligo-epidemiological study of 4134 patients at the National Center for Vitiligo and Psoriasis in Central Saudi Arabia. Saudi medical journal.2011; 32(12): 1291-1296.
20. Akay, B. N., Bozkir, M., Anadolu, Y., & Gullu, S. Epidemiology of vitiligo, associated autoimmune diseases and audiological abnormalities: Ankara study of 80 patients in Turkey. Journal of the European Academy of Dermatology and Venereology. 2010; 24(10): 1144-1150.
21. Sawicki, J., Siddha, S., & Rosen, C. Vitiligo and associated autoimmune disease: retrospective review of 300 patients. Journal of cutaneous medicine and surgery. 2012; 16(4): 261-266.
22. Alkhateeb, A., Fain, P. R., Thody, A., Bennett, D. C., & Spritz, R. A. Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their families. Pigment Cell Research. 2003;16(3): 208-214.
23. Vrijman, C., Kroon, M. W., Limpens, J., Leeflang, M. M. G., Luiten, R. M., Van der Veen, J. P. W., ... & Spuls, P. I.The prevalence of thyroid disease in patients with vitiligo: a systematic review. British Journal of Dermatology. 2012; 167(6): 1224-1235.
24. Nunes, D. H., & Esser, L. M. H.Vitiligo epidemiological profile and the association with thyroid disease. Anais Brasileiros de Dermatologia. 2011; 86(2): 241-248.
25. Kakourou, T., Kanaka-Gantenbein, C., Papadopoulou, A., Kaloumenou, E., & Chrousos, G. P. Increased prevalence of chronic autoimmune (Hashimoto's) thyroiditis in children and adolescents with vitiligo. Journal of the American Academy of Dermatology. 2005; 53(2): 220-223.

Detection of Quorum Sensing Signal Molecules and Identification of *esp*B and Crt4 genes among Biofilm Forming of *Citrobacter freundii*

Wathiq Abbas Hatite Al-Daraghi[1], Mautham Khazaal Kadhim AL-Behadili[1]

[1]*Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq*

Abstract

150 samples from different clinical sources were collected from October 2018 to March 2019 from three hospitals in Maysan: Al Sadr General Education Hospital, Al Zahrawi Surgical Hospital and Maternity and Child Hospital to demonstrate the spread and distribution of *Citrobacter freundii* in several hospitals in Maysan. All isolates were identified based on morphological characteristics and biochemical tests. Results were confirmed by Api 20 E and Vitek 2 compact. A total of 14 isolates (9.3%) of 150 were found to be Citrobacter freundii. The isolates were considered as acute diarrhea in children, UTI, burns, wounds and most frequent ear swabs. PCR results showed that the LuxR 428bp genes were present in the Citrobacter freundii bacteria identified by previous diagnostic methods and this confirms the accuracy of the tests and methods used to determine this type.

Keywords: *Citrobacter freundii; espB and Crt4 genes; Quorum sensing signal*

Introduction

Citrobacter, a genus of the Enterobacteriaceae family, Gram-negative, facultative anaerobic bacteria that look as coccobacilli or rods (1) . Citrobacter spp. are motile using their peritrichous flagella, can ferment mannitol with making of H2S, and can use citrate as their single source of carbon (2),(3). Citrobacter spp. are uncommon opportunistic nosocomial bacteria can cause urinary tract, hematologic, or neonatal infections (e.g. meningitis, sepsis, general bacteremia); intra-abdominal sepsis; brain abscesses; or pneumonia (4) ,(5). Citrobacter spp. infections can be mortal with 33-48% overall death rates being reported including 30% for children(6),(7). Children and immune deficiency, elderly, or weakened patients are at risk of infection (2), (9). Citrobacter spp. is prevailing worldwide, as it is a part of the normal intestinal flora of humans (10),(11). Less well known species that have also been implicated in foodborne disease like some strains of Citrobacter spp. (notably C. freundii), Klebsiella spp., Providencia spp. Enterobacter spp. and Proteus spp., may occasionally cause what is often described as opportunistic gastroenteritis (12), this study aimed to isolation and identification of C. freundii from chicken meat samples using cultural and molecular techniques.

Method and materials

Samples collection

150 samples from different clinical sources were collected from October 2018 to March 2019 from three hospitals in Maysan.

Isolation

each sample was inoculated on the Salmonella shigella (SS) agar medium, the plates were left to solidify at room temperature, and then were incubated at 37 °C for 24-48 hours. Later the grown colonies were further investigated

Identification

The Citrobacter isolates were identified to the level of species using the traditional morphological and biochemical tests [(13)]. The identification of isolates was confirmed by vitek2 compact system.

Cultural characteristics on selective and differential media.

SS, MacConkey and Xylose lysine deoxycholate (XLD) agar

The organisms were cultured on S.S agar media and incubated overnight at 37°C. The colonies of C. freundii appear with black center after 24hrs incubation period, The suspected colonies of C. freundii cultured on MacConky media, the positive result appears pink (Lactose fermenters) after 24hrs incubation period, pale colonies further incubated for 24hrs to identify the (late lactose fermenters). The selected colonies were cultured on Xylose lysine deoxycholate agar, after 24hrs, the positive result appeared as yellow colonies [13].

Eosin Methylene Blue (EMB) agar

In order to differentiate Citrobacter from E.coli, the lactose fermenter isolates were subcultured on EMB for 24hr. at 37Co. Brown colonies were the positive result[14].

Identification of bacteria by Vitek 2 compact system.

Vitek 2 compact was used to identify the bacterial isolates. It is a compact system of two parts, Instrument and computer. The reagent cards have 64 wells that can each contain an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalinisation, enzyme hydrolysis, and growth in the presence of inhibitory substances.

Identification of Bacteria by PCR

DNA Extraction

Genomic DNA was isolated from Bacteria according to the protocol of Wizard Genomic DNA Purification Kit, Intron. A PCR reaction with a specific primer (Table-1).

Table 1-Primers sequences

Primer Name	Sequences	Tm˚C	Size (bp)
LuxR-F	GCACGGATTACATCATTA	49.3	428
LuxR-R	GCACGGATTACATCATTA	49.3	

For **LuxR** gene was performed to identify *C. freundii* (Table-2).

Table 2-Reaction mixture

PCR master mix	Volume
DNA template	5 µL
Green master mix	12.5 µL
Forward Primer10pmol	2.5 µL
Reverse Primer10pmol	2.5 µL
Free nuclease water	2.5 µL
Total	25 µL

(25µl) of PCR amplification mixture contained (12.5 µl) Master mix, (1 µl) forward primer, (1 µl) reverse primer, (8.5 µl) nuclease free water, and (2 µl) DNA template. The protocol for PCR condition was initial denaturation 95°C for 5 min. denaturation 95°C for 30 sec., annealing 60 °C for 40 sec., extension 72 °C for 1 min. and final extension 72 °C for 7min.

Results and Discussion

Bacterial Isolation and Identification

Twenty five chicken meat samples were collected from local markets in Baghdad city. Citrobacter was detected in 3 samples, were all samples cultured on S.S. agar for initial isolation, after incubation at 37°C for 24 hr ; different types of bacterial isolates appeared on S.S. agar, of them: small pale flattened colonies with

black center due to their ability to produces H2S on S.S agar, then these colonies sub-cultured on MacConkey, XLD and EMB to differentiate Citrobacter from Salmonella because both of them are H2S, Citrobacter is lactose fermenter on MacConkey agar appeared as pink colonies while Salmonella is pale colonies (Non lactose fermenter) on XLD Citerobacter appeared as yellow colonies while Salmonella appeared as red colonies with black center . After incubation period; lactose fermenter (pink) on MacConkey and yellow colonies on XLD while on EMB they were brown in colour, these were depended as Citrobacter. To confirm the primary identification Gram stain was performed to examine the microscopic properties which were Gram negative bacilli. The ability of Citrobacter to produce urease enzyme was detected using urease test in order to differentiate it from the genus Proteus which was urease producer while Citrobacter isolates were non urease producers. Thus depending on colonial morphology; bacterial isolates were identified as Citrobacter Figure-1 (A, B, C, D) and (Table-3) showed these biochemical tests used to identify Citrobacter as described by[15], [16].

Figure1-Different selective and differential media cultured with Citrobacter spp. after incubation at 37°C for 24 hr.

A. Pale colonies with black center on S.S. agar

B. Small pink (Lactose fermenter) colonies on MacConkey agar

C. Yellow colonies on XLD agar

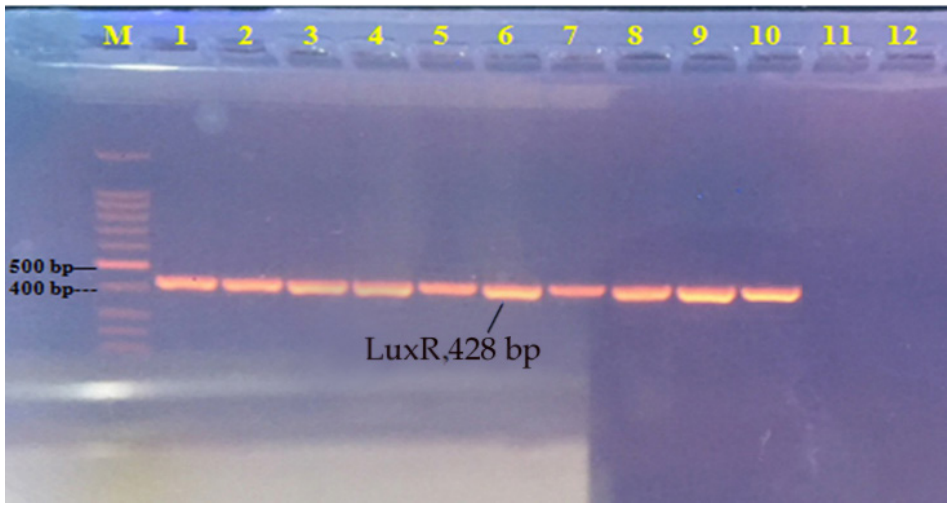
D. Brown colonies on EMB.

Table 3-Result of biochemical tests

Test	Result
Growing on MacConkey agar	Dry Pink colonies
Growing on EMB	Not forms green metallic sheen
Gram stain reaction	Gram negative bacteria
Urease	Non urease producer
S.S agar	Pale colonies with black center
XLD agar	Yellow colonies

To confirm the identification of Citerobacter spp. Vitek 2 compact system was depended and the result showed that the isolated bacteria in this study was Citerobacter and the species freundii

In order to confirm the identification of Citrobacter to species level LuxR gene amplification was performed using monoplex PCR technique, 1.5 % agarose gel electrophoresis was used to detect the positive result as shown in Figure-2.



(Figure 2) Amplified PCR products of LuxR gene (428 bp): Agarose gel electrophoresis, ethedium bromide stained, 1.5 % agarose, electrophoresed in 75 volt for 2 hrs and photographed under ultraviolet trans-illuminator. M: The DNA molecular weight marker (100 bp ladder) and 1: the amplified PCR product of LuxR of C10 isolate of *Citrobacter freundii*.

One of the most gorgeous likely uses of 16Sr RNA gene sequence informatics is to offer genus and species or tax identification for isolates [17]. Although 16SrRNA gene sequencing is highly valuable in regards to bacterial classification [18]. PCR products were exposed to direct sequencing, both strands of PCR products were sequenced with an automatic sequencer. Sequences were analyzed with the Basic Local Alignment Search Tool (BLAST) in National Center for Biotechnology Information (NCBI).

Conclusion

The following conclusions were obtained from this study:

• The prevalence and distribution of Citrobacter spp. in some Maysan hospitals shows a relatively low percentage in its distribution.

• The dominance species of Citrobacter was Citrobacter freundii.

• All isolates of Citrobacter freundii produced Bioflim formation by Congo red agar, Christensen method and micro-titer plate assay.

• The effect of different temperature and pH values on Citrobacter freundii growth showed that best growth temperature was 37°C and the best growth pH for growth was 7.

• These data are of great significance as the signal molecules aid in biofilm formation which in turn confer various properties of pathogenicity to the clinical isolates including drug resistance. The use of quorum sensing signal blockers to attenuate bacterial pathogenicity is therefore highly attractive, particularly with respect to the emergence of multi antibiotic resistant bacteria.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Abbott, S. L. Klebsiella, Enterobacter, Citrobacter, Serratia, Plesiomonas, and other Enterobacteriaceae. In Manual of Clinical Microbiology, 10th Edition, *American Society of Microbiology*. 2011. 639-657.
2. Doran, T. "The Role of Citrobacter in Clinical Disease of Children: Review." *Clinical Infectious Diseases*, 1999. 28(2): 384-394.
3. Knirel, Y., Kocharova N., Bystrova, O., Katzenellenbogen, E. and Gamian, A. Structures and serology of the O-specific polysaccharides of bacteria of the genus Citrobacter. , *rchivum Immunologiae et Therapiae Experimentalis*, 2002. 50(6): 379-392.
4. Thompson, R.,; Perry, J., Stanforth, S. and Dean, J. Rapid detection of hydrogen sulfide produced by pathogenic bacteria in focused growth media using SHS-MCC-GCIMS. *Microchemical Journal*, 2018. 140: 232-240.
5. Ryan, K., Ray, C. and Sherris, J. 2004. Sherris medical microbiology: an introduction to infectious diseases, McGraw-Hill. 237
6. Raphael, E. and Riley, L. W. Infections caused by antimicrobial drug-resistant saprophytic Gram-negative bacteria in the environment. *Frontiers in medicine*, 2017. 4: 183
7. Pepperell, C., Kus, J., Gardam, M. Low-virulence Citrobacter species encode resistance to multiple antimicrobials. *Antimicrob Agents Chemother*, 2002, 46(11): 3555-3560
8. Owoseni, M. and Okoh, A. Assessment of chlorine tolerance profile of Citrobacter species recovered from wastewater treatment plants in Eastern Cape, *South Africa. Environmental monitoring and assessment*, 2017; 189(4): 201.
9. Chen, Y., Wong, W, Fung, C., Yu, K. and Liu, C. Clinical features and antimicrobial susceptibility trends in *Citrobacter freundii* bacteremia. *Journal of microbiology, immunology, and infection*, 2002. 35(2): 109-114.
10. Holmes, B. and Aucken, H. 1998. Citrobacter, Enterobacter, Klebsiella, Serratia and other members of the Enterobacteriaceae, in Collier L, Balows A, Sussman M (eds):Microbiology and Microbial Infections: Systematic Bacteriology (ed 9). London, Arnold, (999-1033).
11. Card, R., Mafura, M., Hunt, T., Kirchner, M., Weile, J., Rashid, M. amd Anjum, M. Impact of ciprofloxacin and clindamycin administration on Gram-negative bacteria isolated from healthy volunteers and characterization of the resistance genes they harbor. *Antimicrobial agents and chemotherapy*, 2015. 59(8): 4410-4416.
12. Lawley, R., Curtis, L. and Davis, J. 2012. The food safety hazard guidebook. *Royal Society of Chemistry*.
13. MacFaddin, J. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams and Wilkins, Philadelphia, PA.
14. Bettelheim, K., Evangelidis, H., Pearce, J., Sowers, E. and Strockbine, N. A. Isolation of a *Citrobacter freundii* strain which carries the *Escherichia coli* O157 antigen. *Journal of clinical microbiology,* 1993. 31(3): 760-761.
15. Janda, J., Abbott, L., Cheung, W. and Hanson, D. Biochemical identification of Citrobacteria in the clinical laboratory. *Journal of Clinical Microbiology,* 1994. 32(8): 1850-1854
16. Gwida, M., Hotzel, H., Geue, L. and Tomaso, H. Occurrence of Enterobacteriaceae in raw meat and in human samples from Egyptian retail sellers. *International scholarly research notices*. 2014. (2014):1-6.
17. Janda, J. and Abbott, S. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *Journal of clinical microbiology*, 2007. 45(9): 2761- 2764.

18. Bosshard, P., Zbinden, R., Abels, S., Böddinghaus, B., Altwegg, M. and Böttger, E. "16S rRNA gene sequencing versus the API 20 NE system and the VITEK 2 ID-GNB card for identification of nonfermenting Gram-negative bacteria in the clinical laboratory." *Journal of clinical microbiology*, 2006. 44(4): 1359-1366.

The effect of Beta-amino Butyric Acid in Levels of Interleukin4 & Interleukin 10, Complement Proteins C3 & C4 and Immunoglobin IgM in Males Rats Sprague Dawley Infected with *Pseudomonas aeruginosa*

Zainab Makki Sagheer[1] Mohammad Abas Jasim[1]

[1]Department of biology/ College for Women Education University of Anbar/Iraq

Abstract

B–amino butyric acid (BABA) non protein amino acid has effect on some immunological parameters of male rats Sprague Dawley species infected with *pseudomonas aeruginosa.* this study include 25 animal divided into five groups A, B, C, D and E each group contain five animals, A, B and C groups injected with three concentration of amino acid solution (25 mg\ml, 50 mg\ml and 75 mg\ml) with dose (0.2 g\ kg, 0.4g\kg and 0.6 g\kg) respectively, intra peritoneal weekly for six weeks, group D (first control group) and E (second control group) injected with normal saline. At the fifth week the four groups A, B, C and D exposed to *pseudomonas aeruginosa,* while group E did not exposed to infections after the termination of period of treatment, The blood samples were collected to do the immunological tests, the statistical analysis $P<0.05$ results showed BABA did not enhance sensitivity through measuring interleukin IL4, and significant increase of IL10 and complement protein C4, while BABA didn't have a negative effect on the complement protein C3 activation and increase the level of immunoglobulin IgM.

Keywords: *Beta-aminobutyric acid, Sprague Dawley rats, Pseudomonas aeruginosa, Interleukin IL10, IL4, Complement proteins C3 & C4, Immunoglobin IgM.*

Introduction

The bacteria considered important causes of disease, that include the most of diseases that infect humans in the word and the most important prefix and present and futurism epidemiological diseases[(1)], *Pseudomonas aeruginosa* considered important bacterial species due to its content of virulence factor that increase its pathogenicity[(2)]. It's considered dangerous for patients especially patients with wounds & burn inflammation, that it can invade blood supply and cause Septicemia especially patients with Immunodeficiency[(3)]. *P. aeruginosa* characterized by its resistance to antibiotics and disinfectant, and this cause big problem in whole the word and the main cause of this resistance is random use of antibiotics without restriction[(4)], due to continues antibiotic residue lead to development of bacterial resistance to antibiotics[(5)], this what make researchers to new applications by using chemical materials other than antibiotics[(6)], that enhance body resistance to bacterial infections, the nominated materials to replace antibiotics is organic acids[(5)], Which are include many acids like Non-protein amino acids that present in the nature, more than 1000 non protein amino acid produced by plants and microorganism and other sources[(7)], these components did not have specific functions in the nature, but they noticed have many physiological functions *In vivo* studies[(8)], For the example, Gamma-amino butyric acid (GABA) work as stimulator and regulator of immune response that it boosted the Innate immunity and Adaptive immunity, that work on improvement of body resistance against bacteria by activation of Phagocytosis, and matureness of Macrophages and improve its response against microorganism[(9)], and activation of T cells and B cells, increase antibodies like IgG and IgM[(10)], GABA increase of Anti-inflammatory cytokines like interleukin IL10 and suppression of Pro-inflammatory cytokines and work on stimulation of Apoptosis of cells that damaged by pathogenic factors[(11)], also the Non-protein amino acids β-amino butyric acid (BABA) was improved recently that make hematological and immunological changes, that increase

red blood cells, white blood cells, hemoglobin, packed cells volume and lymphocyte, also cause increase in immunoglobulin IgG[12].

Due to rarity of studies about the effect of this acid in animal aspect with its availability in plant aspect, and due to BABA known with its ability to stimulate plant resistance against wide range of causative agent like viruses, bacteria, fungi and worms[13], also have significant effect in plant resistance to insects[14].

So this study aimed to identification of ability Non-protein amino acids BABA to induction of rats resistance to *P. aeruginosa.*

Materials and Method

Preparation of amino acid solution

Amino acid solution prepared by dissolving 0.5g, 1g and 1.5g from acid in 20ml of normal saline to obtain the first concentration C1 (25mg\ml) and second concentration C2 (50mg\ml) and third concentration C3 (75mg\ml) respectively with continues mixing until acid dissolving then the wanted dose prepared according to animal body weight, with percentage of 0.2g\kg from first concentration C1 and 0.4g\kg from second concentration and 0.6 g\kg from third concentration.

Preparation of the lab animals

Experimental animals consist of 25 of Sprague Dawley male rats with age 10 to 12 weeks and weight ranged between (225-300) g, divided into five groups each groups five animals divided as following:

1- Group A first concentration group C1(25mg\ml)

2- Group B second concentration group C2 (50mg\ml)

3- Group C third concentration group C3 (75mg\ml)

The groups above injected with amino acid intrapersonal[15] weekly for six weeks.

4- Group D first control groups: injected with normal saline intrapersonal.

The four groups above exposed to infection with *P. aeruginosa* Bactria.

5- Group E second control group this group injected with normal saline intrapersonal and did not exposed to infection with *P. aeruginosa*.

Animal infection with *P. aeruginosa*

The animal infected after the fifth weeks of amino acid BABA injection at age (15-17) weeks with weight ranged between (265-380) g. the isolation activated with by obtain part of bacterial cultivation to inoculate nutrient broth tube and incubate with 37 C for 24 hours. After appear of the bacterial growth decimal dilution done by using normal saline and 1 ml of each diluent was obtained and inoculates on nutrient agar to count the colonies in the suspension. Rats anesthetized with Chloroform. Than shaved the area down the head from the back[16]. Wounded the skin deeply without damage the subcutaneous muscles, after sterilization by using forceps and Scissor, skin biopsy obtained in about 6mm diameter of five animal groups (A, B, C,D and E), by using Micropipette 200µm of suspension with turbidity (2×10^6) putted on the wound to contaminate it[17] in four animal groups that include group A, group B, group C and first control group D, while second control group E did not contaminated with bacteria.

The animals examined for its nutrition and activity and wound healing after expend of seven days of infections the physiological and immunological tests were done.

Serological tests

Immunological tests were carried out on the serum of the rats to detect the level of IL-4, IL-10, C3, C4 and IgM in all groups. Blood was obtained from posterior vena cava[15].

3 ml of blood was withdrawn in plastic tubes free of anticoagulant to obtain the serum using centrifuge 3500 rpm for ten minutes. The serum samples were kept at -20°C until the time of testing using (Elisa kit/ Elabscience Biotechnology Inc. /USA) and (Genus Kit/ Genrui Biotech Inc./China)

Statistical Analysis

The statistical analysis done by using One-way ANOVA test with statistical analysis program SPSS 22 edition and the mean calculated and stander deviation, finding LSD value from Multiple comparisons table at level of significance 0.05.

Results and Discussion

Effect of BABA on level of interleukin IL4

The results of statistical analysis as in table 1 which show the significant differences in LSD values between groups, and figure 1, show that A, B, C and D (three concentration group and first control group) that exposed to bacterial infection was recorded significant increase in level of interleukin IL4 compare with second control group that did not exposed to bacterial infection.

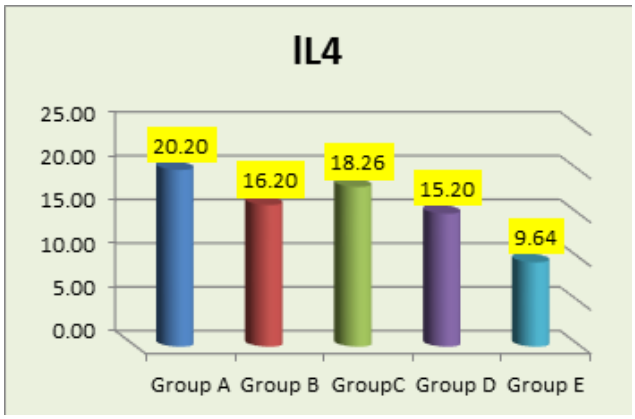


Figure 1 show the means of interleukin IL4 values

From the above the level of IL4 that increased in infected groups as the results of infection with bacteria not resulted from BABA amino acid that appeared the mast cells are activated to interact with wound repairing and wound healing at the same time with infection by microorganism, that have vigilant factors that disturb wound healing mechanism as Exotoxins of *P. aeruginosa* this cells stimulated presented in the subcutaneous tissue that migrate to the wound site and secrete its content due to expose to bacteria products, the studies conclude that the mast cells activated by its exposer to bacterial toxin or lipopolysaccharide (LPS) bacterial wall content, also fined that the mast cells that present in peritoneal layer execrate Histamine as the results of *P. aeruginosa* bacteria in rats, and it's an important source of early response cytokines like IL4 that necessary to begin the immune response and inflammation of the host ageist invaders[18], and considered main resource of IL4[19], that the mast cells activated by bacteria that cause diseases also in the case of absence of antibodies and information and in this case be the source to generate IL4 and other regulating cytokines to do Non-opsonization reactions[18], the mast cells response to many stimulators independently without interaction of IgE and release its component, in this situation did not considered hypersensitivity response because IgE not unclouded in the interaction, and this explain support absence of signs of hypersensitivity in animals.

Effect of BABA on level of IL10

The results of statistical analysis $P<0.05$ as in table 1 and figure 2 show the A group record significant increase in level of IL10 in compare with E and D control groups, also group C record showed significant increase in level of IL10 in compare with E second control group.

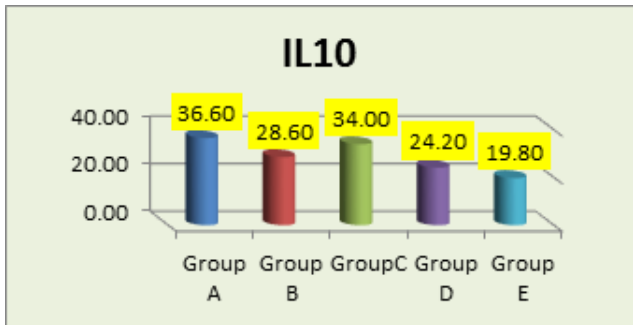


Figure 2 show the means interluken 10 value

By notest the figure 2 showed that the mean of IL10 value elevated in animal groups treated with BABA amino acid in compare with E and D control groups but just group A recorded significant increase in compare with control groups E and D, and group C recorded significant increase in compare with E second control group, while group B did not recorded any significant increase for unclrear causes, and this may explane the level of IL10 in group A and the dose 0.2 g\kg of BABA amino acid and this dose needed for production of IL10 with significant level and this agree with what mentioned by[11], that the GABA which analogous with BABA, increase the production of IL10 significantly in case of colon inflammation induced in mice, that GABA work as stimulator for antiinflammatory cytokines and inhibit Pro-inflammatory cytokines and enhance Apoptosis for cells that damaged with diseases factors.

Effect of BABA levels of complement proteins C4 and C3 and immune globulin IgM

Results of Statistical Analysis $P<0.05$ showed as appeared in table 1 and figure 3 that the five group A, B, C, D and E did not show any significant increase in level of complement protein C3, with non-significant sharp decrease of C3 mean values in group A, also decrease the value of C3 in second control group E, and the decreased values indicate the activation of complement protein[20].

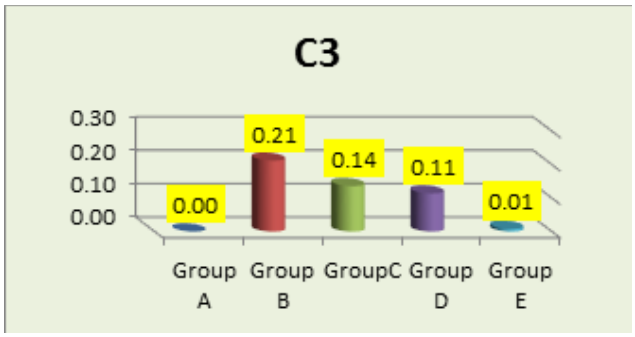


Figure 3 show the means of complement protein C3 values

While the complement protein C4 the results of statistical analysis P<0.05 were showed as in table 1 and figure 4 that the group A recorded significant increase in level of C4 in compare with group B, while other groups did not recorded any significant differences, and this increase may cause by bacterial infections or may be due to the 0.2 g\kg dose of BABA acid is the optimal dose to increase complement proteins C4 significantly, as appear in figure 4 that showed means of complement proteins C4 values.

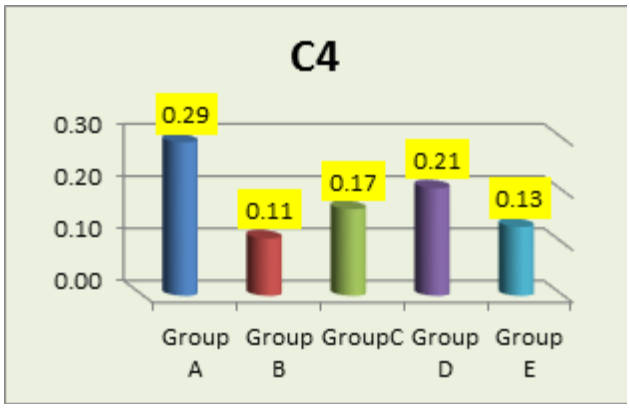


Figure 4 show the means of complement protein C4 values

From the compares between figure 3 and figure 4 noticed there are negative correlation between C3 and C4 values and this difference may return to activation of complement C3 to activate Alternative pathway or Lectin pathway as an innate immune response to infection, and lead to activation B cells to production of antibodies to activation of Classical pathway[21]. And this indicates that the BABA amino acid did not have negative effect to prevent complement C3 activation process. And have positive effect in activation of classical pathway of complement system that depends on C4 protein.

While measurement of immunoglobulin IgM, the results of statistical analysis (P<0.05) as in table 1 and figure 5, that the group A was recorded significant increase in level of IgM in compare with group B, C and E, that the 0.2 g\kg lowest dose suitable to production of IgM and this results did not agree with[21] mentioned that the increase dose increase production of immunoglobulin IgG and this may returned to interactions with bacterial infections or may results category of antibody and molecular compound of each one.

Figure 5 show the means of immunoglobulin IgM values

By compare of figure 4 and figure 5 noticed that presence of clear positive proportions between complement protein values C4 and immunoglobulin IgM, and this may lead to activation classic pathway of complement system, the interaction of B cells that produce immunoglobulin IgM that stimulate activate of classic pathway of complement system, and activation

Table 1 show the LSD values

Dependent Variable		IL4	IL10	C3	C4	IgM
Group A	Group B	.146	.166	.113	.048*	.003*
	Group C	.471	.646	.300	.158	.006
	Group D	.073	.038*	.383	.326	.105
	Group E	.001*	.007*	.951	.079	.008*
Group B	Group A	.146	.166	.113	.048*	.003*
	Group C	.445	.344	.559	.529	.737
	Group D	.709	.439	.452	.285	.103
	Group E	.022*	.130	.126	.804	.748

Cont... **Table 1 show the LSD values**

Group C	Group A	.471	.646	.300	.158	.006*
	Group B	.445	.344	.559	.529	.737
	Group D	.260	.094	.885	.652	.187
	Group E	.004*	.019*	.328	.701	.989
Group D	Group A	.073	.038*	.383	.326	.105
	Group B	.709	.439	.452	.285	.103
	Group C	.260	.094	.865	.652	.187
	Group E	.048*	.439	.417	.407	.183
Group E	Group A	.001*	.007*	.951	.079	.006*
	Group B	.022*	.130	.126	.804	.748
	Group C	.004*	.019*	.328	.701	.989
	Group D	.048*	.439	.417	.407	.103

*means difference is significant in LSD values between groups at the 0.05 level

Conclusions

Current study insure that the non-protein amino acid BABA have clear positive effect in accelerate of wound healing process and decrease inflammation, the serological tests P<0.05 ensure that the BABA did not generate hypersensitivity in animal of experiment. BABA have positive effect in production of IL10 then it has role in regulation of immune response, BABA did not stop activation of complement system but lead to stimulation of B cells to production of IgM antibody and inter action between Innate immunity and Adaptive immunity.

Acknowledgement: I would like to thank Dr Othman Makki Sagheer and Dr Atheer Khalaf

for their scientific support for this research. I would also thank Dr Yasser Mufeed and Ms. Anwar Yousef for their efforts to complete this research.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1- Philips JA, Blaser MJ. Introduction to bacteria and bacterial Diseases.Mandle, Douglas and Bennetts principles and practice of infectious diseases. Elsevier Inc. (2015);8(2):2236-2234.

2- Neamah, AA. Molecular detection of virulence factor gene in *Pseudomonas aeruginosa* isolate from human and animals in Diwaniya province Kufa. J. Vet. Med. Sci. (2017);8(1):218-231.

3- Jalil MB, Abdul-Hussein ZR, Al-Hmudi HA. Isolation and identification of multi drug resistant biofilm producer with burn wound infection in Basra province/Iraq. IJDR. (2017);7:11.

4- Mitiku M, Ali S, Kibru G. Anti-microbial drug resistance and disinfectants susceptibility of *Pseudomonas aeruginosa* isolate from clinical and environmental samples in Jimma University Specialized Hospital. South West Ethiopia. AJbls. (2014);2(2):40-45.

5- Panda AK, Rama RV, Raju MV, Shyam-Sunder G. Effect of butyric acid on performance, gastrointestinal tract health and Carcass characteristics in Broiler chickens. Asian-Aust. J. Anim. Sci. (2009);22(7):1026-1031.

6- Yang P, Iji PA, Choct M. Effect of different dietary levels of mannan oligosaccharide on growth performance and gut development of Broiler chickens. Asian-Aust. Anim. Sci. (2007);20:1084-1091.

7- Barret, G. Chemistry and Biochemistry of the Amino Acids. Chapman and Hill. London. (1985).

8- Sahyan AS, Langer P. (2016). Asymmetric synthesis of non-proteinogenic amino acid. 1st ed. Wiley Verlag GmbH & Co. KGaA. (2016);8-9.

9- Kim JK, KimYS, Lee H, Jin HS, Neupane C, Kim S, Lee S, Min J, *et al*. GABAergic signaling linked to autophagy enhance host protection against intra cellular bacterial infection. Nat. Commun. (2018);9:4184.

10- Tang J, Chen Z. The protective effect of *y*-aminobutyric acid on the development of immune function in chickens under heat stress. Wiley online Library, Journal of Animals Physiology and Animals Nutrition. (2016);100(4):768-777.

11- Ma X, Sun O, Sun X, Chen D, Wei C, Yu X, Liu C, Li Y, Li J**.** Activation of GABA receptors in colon epithelium exacerbates acute colitis. Front Immunol. (2018);9:987.

12- Jassim MA, Saleh EN. Effect of intraperitoneal verus oral drench of beta-aminobutyric acid on hematology and immunoglobulin in Spraque Dawely rats. Onl J Vet Res. (2018);22(9):784-788.

13- Justyna PC, Ewa K. Induction of resistance against pathogens by B-aminobutyric acid. Actaphy Siologiae Plantavum. (2013);35:1735-1748.

14- Tiwari S, Meyer WL, Stelinski L. Induction resistance against the Asin citrus *Psyllid, Diaphorina citri* by B-aminobutyric acid in citrus. Bull Entomol Res. (2013);103:592-600.

15- Perret-Gentil M I. Rat biomethodology. university veterinarian & director. laboratory animal resources center.the university of texas at san antonio. (2010);210:458-6173.

16- Deleon K, Trivedi U, Griswold JA, Lyte M, Hampel KJ, *et al. Pseudomonas aeruginosa* Biofilms perturb wound resolution and antibiotic tolerance in diabetic mice. Med Microbiol Immunol. (2013);202(2):131-141.

17- Kanno E, Toriyabe S, Zhang L, Imai Y, Tachi M. Biofilm formation on rat skin wounds by *Pseudomonas aeruginosa* carrying the green fluorescent protein gene. Experimental Dermatology. (2010);19:154-159.

18- Abraham SN, Malavia R. Mast cell in infection and immunity. Infection and Immunity. (1997):301-3508.

19- Bradding P, Okayama Y, Howarth PH, Church MK, Holgate ST. Heterogeneity of human mast cells based on cytokine content. J. Immunol. (1995);155:297-307.

20- Hussain N, Jaffery G, Hasnain S. Serum complement C3 and C4 level in relation to diagnosis of Lupus nephritis. Tropical Journal of Pharmaceutical Research. (2008);7(4):1117-1121.

21- Saleh AN. Study the variations resulting from the effect of non-protein amino acid Beta-amino butyric acid (BABA) in rats male cells Sprague Dawley. Mcs Thesis, Collage of women, University of Anbar. (2019).

The Toxic Effect of Cadmium Chloride on Lung Function and Tissue and the Protective Role of Pomegranate Seed Oil in Female Rabbits

Noor Ibrahem Hasan AL-Kraie[1], Israa Salman Dalas[1]; Qasim Azeez Razooqi[1]

[1]College of Education for Pure Sciences, Tikrit University, Tikrit, Iraq.

Abstract

The experiment was designed to determine the protective role of pomegranate seed oil on lung function and tissue against cadmium chloride toxicity.

In this experiment, 20 animals (rabbit females) were used. Lung efficiency was measured by measuring the size of red blood cells and hemoglobin, as well as studying the histological effects of cadmium chloride against lung tissue and the protective role of pomegranate seed oil . The results showed that the treatment of animals with cadmium chloride at a dose of 5 mg / kg bw for 30 days led to a significant increase in packed cell volume (PCV) and hemoglobin (Hb) compared to control group .

The treatment of animals with cadmium chloride has also shown that there is no possibility of distinguishing the alveolar of lung (AV) with the presence of focal infiltration of the inflammatory cells with a marked thickening of the wall of the blood vessel (TW) with blood congestion (CON) Pomegranate seed oil improves most of the negative effects caused by cadmium chloride .

Key word : *Cadmium chloride , Pomegranate seed oil , Packed cell volume (PCV), Hemoglobin (Hb) .*

Introduction

Cadmium (Cd) and lead (Pb) are ubiquitous and non-biodegradable pollutants representing a great concern to human health. Both metals are naturally distributed, but industrial development has dramatically increased their concentrations in the environment [(1).] The World Health Organization (WHO) has published a list of 10 chemicals or groups of chemicals of concern for human health, which includes Cd [(2).] The toxicity of cadmium has attracted the attention of researchers in different countries of the world due to its toxic effect on the cells and tissues of the body. The focus of the researchers has been on reducing the effect of effective oxygen species and free radicals causing their formation, as well as increasing interest in reducing the impact of industrial pollutants in the environment in which we live [(3)]. So researchers have sought to use a large number of plant-derived pharmaceutical products in traditional medicine or to use their extracts because of their useful properties. The plants are rich in a wide range of secondary compounds such as flavonoids, tannins, alkaloids and others [(4)] . Mentioned that the damaged central nervous system and DNA or cancer progression appeared as consequences of Cd exposure. Cadmium also causes severe soft tissues and bone damages [(5).] After absorption, Cd and Pb are distributed in the organisms via red blood cells or proteins . Amajor amount of Cd in red blood cells is bound to high-molecular-weight proteins, while a minor amount is bound to hemoglobin [(6)] . The hematopoietic system is one of the most sensitive systems and blood represents not only the mode of transportation, but also the critical toxicity target of Cd and Pb [(7).] Cadmium causes lung damage, pulmonary fibrosis, emphysema, and inflammation in human and experimental animals. Cadmium may also adversely affect the lungs by decreasing the viability or modifying the function of individual lung cells [(8).] The toxic mechanisms responsible for cadmium-induced lung cell damage are not well understood. One study [(9)] .

The use of extracts from medicinal plants and their effective non-food chemical compounds has a preventive and therapeutic effect for many disease cases and has little or no side effect compared to chemically manufactured laboratory drugs [(10)]. Pomegranate, Punica granatum L.,

is an ancient medicinal food plant which natively grows from the Himalayas in northern India to Middle East but has also been cultivated and naturalized in many other regions including Mediterranean, Southeast Asia, tropical Africa, and American Southwest [11]. In addition to extensive uses of pomegranate in folk medicine of many cultures, pharmacological studies have shown that pomegranate fruit preparations have antioxidant and anti-inflammatory,[12] antimicrobial,[13] anticancer, and chemopreventive [14] . Pomegranate seeds are rich in sugar, unsaturated- polyunsaturated fatty acids, vitamins, polysaccharides, polyphenols and minerals [15]. In particular, pomegranate seed oil contains high levels of phenolic compounds which is punicic acid, punicalagins (PNG), as well as important fatty acids such as linoleic acid, gallic acid and elagic acid [16] . Ellagic acid is a polyphenol compound with antioxidant and anti-proliferative properties that also exists in many other fruits and plants such as raspberries, pecan nuts and strawberries. ese components demonstrate anti-inammatory and antioxidant eects by inhibiting the expression of pro-inammatory enzymes and cytokines [17]. anticarcinogenic , antioxidant [18] anti-inammatory , antimicrobial [19, 20]. which are free radical scavenging compounds [21] Pomegranate is also rich in vitamins and minerals [22].

Material and Method

2-1-Experimental Design: After acclimatization, animals were randomly divided into four groups: Group 1 –Animals were given distilled water and kept as control.

Group 2 – Animals were given Pomegranate seeds oil (0.8 ml)/ kg b.w. for 30 days [23] .

Group 3- Female rabbits were treated at a dose of cadmium chloride 6 mg/kg b.w for 30 days, which promised an infected control. [24] .

Group 4 – Animals were given Pomegranate seeds oil (0.8 ml)/ kg b.w. with cadmium chloride 5 mg/kg b.w for 30 days .

2.2. Hematological Examination:

Blood samples were taken from the retroorbital venous plexus of rats. The two blood samples were collected one with EDTA for hematological analysis and other for separate serum for biochemical analysis. Erythrocyte count (RBCs) was performed using improved Neubauer Hemocytometer and Gower,s fluid as a diluting fluid according to (25). PCV% was determined by using microhematocrite centrifuge and microhematocrite capillary tubes method according to [26].

2.3. Histological study

Lung tissue samples were fixed in 10% formalin since 24 hours, dehydration by ethyl alcohol in increasing concentrations (70%, 80%, 95%, 100% and100%), clearing with xylene and then embedded with paraffin. When analyzed, all paraffinembedded tissue was sectioned at 5 µm ,and stained with Hematoxilin and eosin. These specimens were examined under a light microscope at 40X magnification power. Corresponding digital images were captured for later analysis [27] .

Results

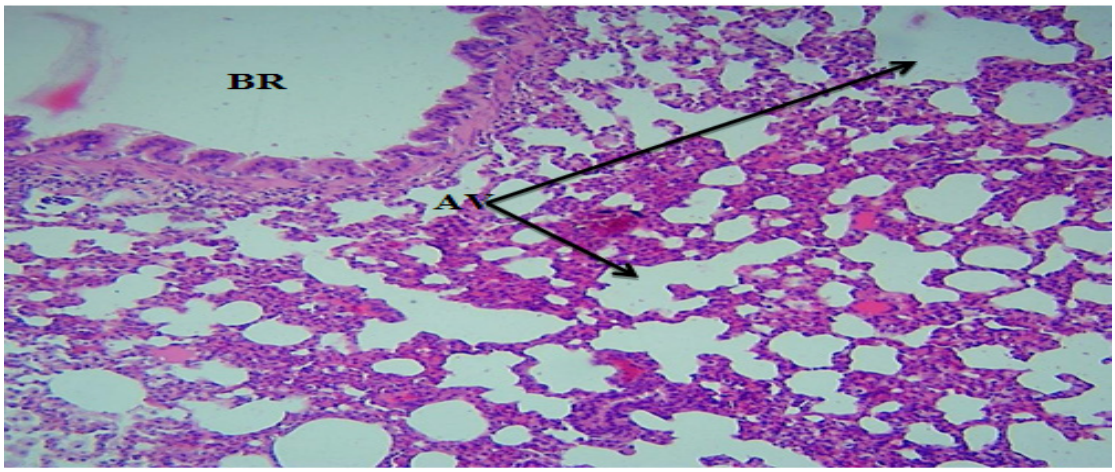


Figure (1) The lung segment control group shows pulmonary bronchioles (BR) and the alveoli (AV) within the lung tissue in its natural form H & E 400X

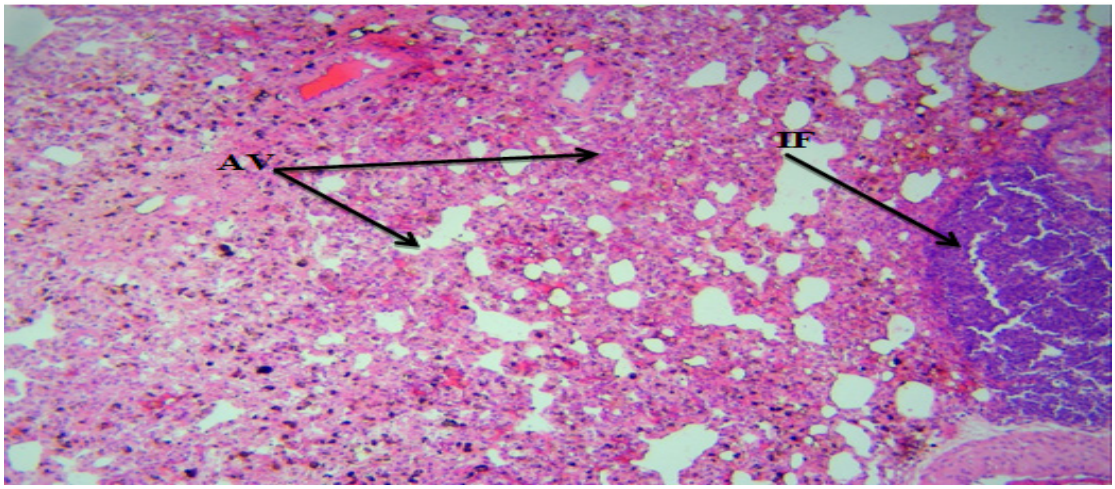


Figure (2) The lung segment The treatment of cadmium chloride shows that it is not possible to distinguish alveoli (AV) with focal infiltration of inflammatory cells (IF) H & E 100X

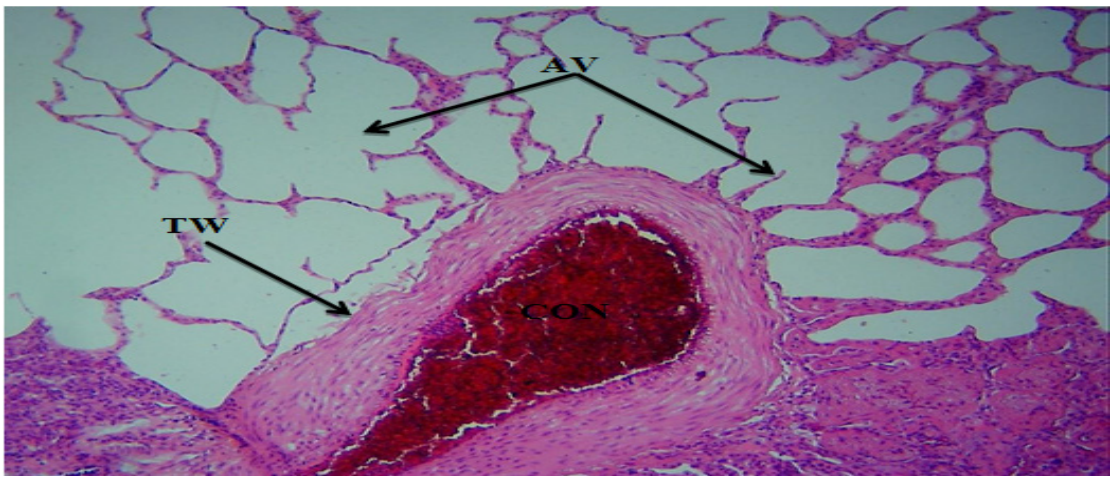


Figure (3) The lung section The cadmium chloride treatment shows the renal variability (AV) with a marked thickening of the wall of the blood vessel (TW) with blood congestion (CON) H & E 100X

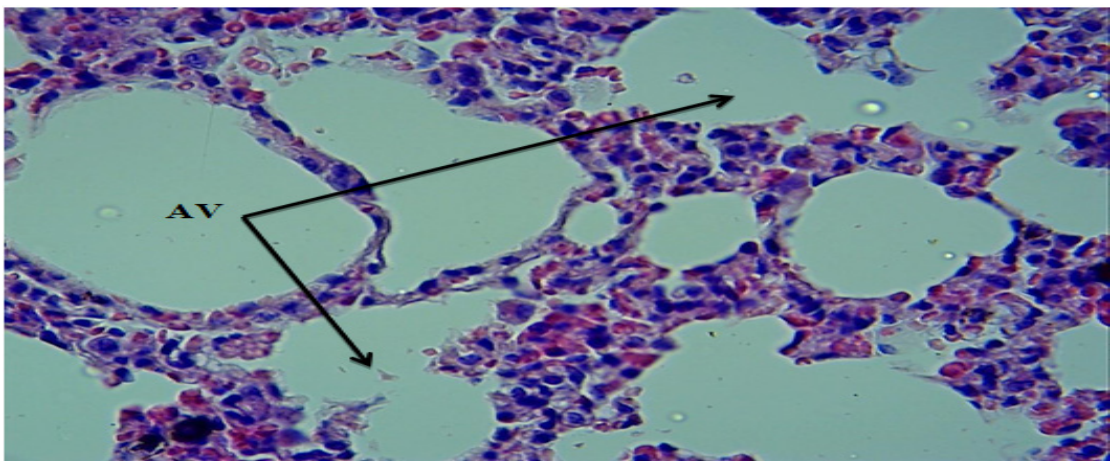


Figure (4) Lung section group treated with pomegranate seed extract showing normal the alveoli (AV) H & E 400X

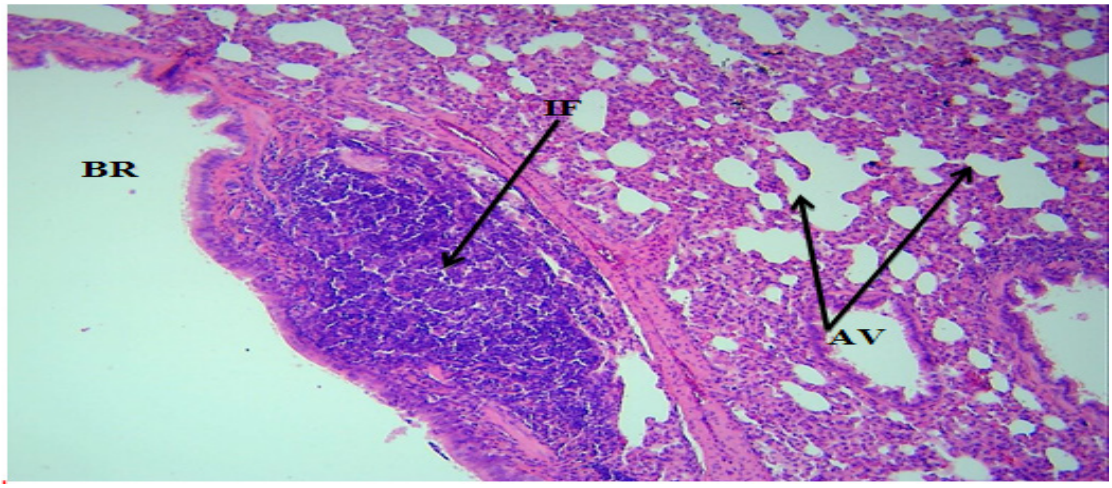


Figure (5) The lung of the cadmium chloride and pomegranate seed oil shows the the alveoli (AV) with a central infiltration of inflammatory cells (IF) adjacent to the bronchioles (BR) H & E 100X

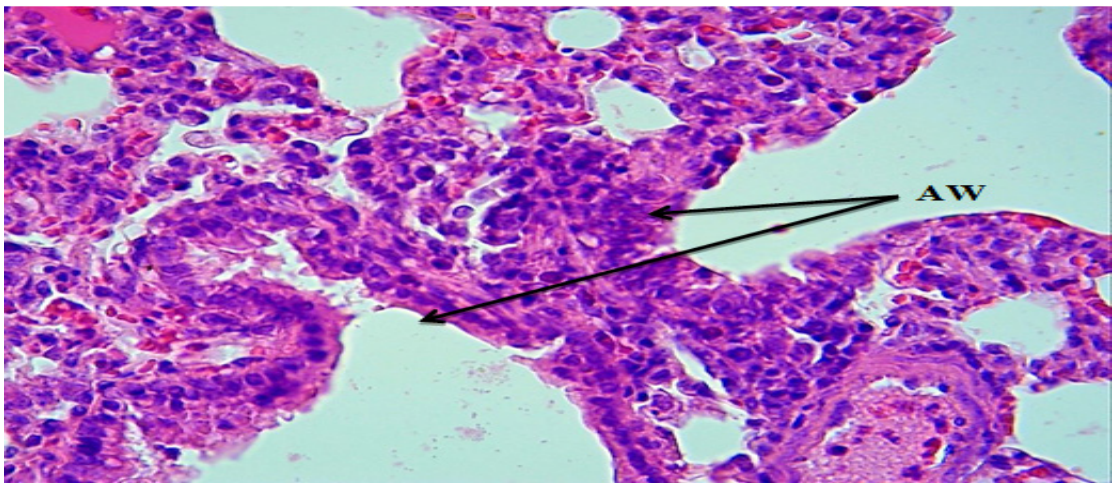


Figure (6) Lung section of the treated group Cadmium chloride and pomegranate seed oil shows the thickening of the (AW) wall clearly H & E 400X

Discussion

It is noted from the results above that treatment of animals with cadmium chloride at a dose of 6 mg / kg body weight for 30 days led to significant increase ($P \leq 0.05$), in blood volume and hemoglobin compared to control group, in consonance with found . [28] . While did not agree with [29], Who used a single dose with cadmium chloride (0.1mg / kg) . It was found that the treatment of mice With cadmium for 21 days led to a significant decrease in the value of Hb . while the protective role of pomegranate seed oil against chloride Showed a significant decrease in blood volume and hemoglobin compared with the cadmium chloride-treated animals group . The hematopoietic system is one of the most sensitive systems and blood represents not only the mode of transportation, but also the critical toxicity target of Cd and Pb. [7]. Both metals may lead to anemia by various mechanisms [30]. Cadmium and Pb are transported to the liver, in which they can cause damage and disturbed function. Liver damage can be confirmed by histopathological findings and is often accompanied by increased blood enzyme levels and reduced protein synthesis [31].

Changes in the size of red blood cells The cause is believed to be a physiological condition to compensate for the lack of oxygen in the body because of the thickening of the gas exchange membrane between the alveolar of lung and the blood and these changes lead to increase the formation of red blood cells from the reservoir of body [32] . This problem is also very important and interesting because there are many reports in literature that cadmium can result hypoxia [33]. That's why it is topical to research metabolical effect of cadmium ions and hypoxia and find out the biochemical and morphological changes of

blood indices of rats under cadmium loading as blood is a substance of organism that reacts on irritation from environment very quickly.

The decrease in RBCs count during the chronic treatment might be resulted from severe anemic state or haemolysing power of heavy metals (cadmium chloride) particularly on the red cell membrane. this agreed with [34] . The reduction in erythrocytes count might be due to the destruction of mature erythrocytes and the inhibition of erythrocytes production . It is also noticed from the tissue sections of the lung tissue that the treatment of animals with cadmium chloride led to the possibility of distinguishing the pneumonia (AV) with the presence of central infiltration of inflammatory cells with a clear thickening in the wall of the vessel (TW) with congestive blood (CON), in consonance with[35,36].

Lung tissue is one of the main targets of cadmium toxicity [37], and the respiratory system is affected severely by the inhalation of cadmium-contaminated air. Shortness of breath, lung edema, and destruction of mucous membranes as part of cadmium-induced pneumonitis have been described. Cadmium causes lung damage, pulmonary fibrosis, emphysema, and inflammation in human and experimental animals. Cadmium may also adversely affect the lungs by decreasing the viability or modifying the function of individual lung cells [8] .

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1) Satarug, S.; Garrett, S.H.; Sens, M.A.; Sens, D.ACadmium, environmental exposure, and health outcomes. Environ. Health Perspect. .(2010). 118, 182–190.

2) WHO. Action Is Needed on Chemicals of Major Public Health Concern. Public Health Environmental, (2010). pp. 1–4.

3) Prasad, S.; Gupta, S.C.; Tyagl, A.K . and Lokesh, D. Reactive oxygen species and cancer . Role of antioxidative nutria ceuticals . Cancer letters, 2017 Feb 28;387:95-105.4)Ghiasi Ghalehkandi, J.; Ebrahimnezhad, Y.; Maheri Sis, N. The effect of aqueous garlic extract and chromium chloride complement on tissue anti oxidant system of male rats. J Anim Plant Sci, (2013). 23:56-59.

5) Cucu D, D'Haese PC, De Beuf A, Verhulst A Low doses of cadmium chloride and methallothionein-1-bound cadmium display different accumulation kinetics and induce different genes in cells of the human nephron. Nephron Extra (2011). 1(1):24-37.

6) Timchalk, C.; Lin, Y.; Weitz, K.K.; Wu, H.; Gies, R.A.; et al. Disposition of lead (Pb) in saliva and blood of Sprague-Dawley rats following a single or repeated oral exposure to Pb-acetate. Toxicology, (2006). 222, 86–94.

7) ATSDR (Agency for Toxic Substances and Disease Registry). Toxicological Profile for Cadmium; The United States Public Health Service, Agency for Toxic Substances and Disease Registry: Atlanta, GA, USA; (2012). pp. 1–487.

8) Lag M, Westly S, Lerstad T, Bjornsrud C, et al.. Cadmium-induced apoptosis of primary epithelial lung cells: involvement of Bax and p53, but not of oxidative stress. Cell Biol. Toxicol. (2002)18: 29-42.

9) Kostial K, Kello D, Jugo S, Rabar I, et al. Influence of age on metal metabolism and toxicity. Environ. Health Perspect. (1978). 25: 81-86.

10) Krishnaiah, D.; Sarbatly, R. and Nithyanandam, R.R .(2011) . A review of the antioxidant potential of medicinal plant species , Food and Bioproduct processing, (1978). 89:217-233.

11) Jurenka JTherapeutic applications of pomegranate (Punica granatum L.): A review. Altern Med Rev. .(2008) 13(2):128–144.

12) Lansky P, Newman RAPunica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J Ethnopharmacol. .(2007) 109:177–206.

13) Huang TH, Peng G, Kota BP, et al. Antidiabetic action of Punica granatum flower extract: activation of PPAR-gamma and identification of an active component. Toxicol Appl Pharmacol.; (2005). 207:160–169.

14) Adams L, Seeram NP, Aggarwal BB, Takada Y, Sand D, Heber D. Pomegranate juice, total pomegranate tannins and punicalagin suppress inflammatory cell signaling in colon cancer cells. J

Agric Food Chem. (2006). 54:980–985.

15) Afaq F, Saleem M, Krueger CG, Reed JD, Mukhtar H. Anthocyanin- and hydrolyzable tannin-rich pomegranate fruit extract modulates MAPK and NF-kappaB pathways and inhibits skin tumorigenesis in CD-1 mice. Int J Cancer; (2005). 113:423-433.

16) Xu J, Guo CJ, Yang JJ, Wei JY, Li YF, Pang W, et al. [Intervention of antioxidant system function of aged rats by giving fruit juices with different antioxidant capacities]. Zhonghua Yu Fang Yi Xue Za Zhi; (2005). 39:80-83.

17) Rasheed Z, Akhtar N, Anbazhagan AN, Ramamurthy S, et al. Polyphenol-rich pomegranate fruit extract (POMx) suppresses PMACI-induced expression of proinammatory cytokines by inhibiting the activation of MAP kinases and NF-kappa B in human KU812 cells. Journal of Inammation; .(2009) 6: 1–30.

18) Bouroshaki MT, Sadeghnia HR, Banihasan M, Yavari S. Protective eect of pomegranate seed oil on hexachlorobutadieneinduced nephrotoxicity in rat kidneys. Renal Failure; (2010). 32: 612-7.

19) Braga LC, Shupp JW, Cummings C, et al. Pomegranate extract inhibits Staphylococcus aureus growth and subsequent enterotoxin production. J of Ethnopharmacology; (2005). 96:335– 9.

20) Menezes SM, Cordeiro LN, Viana GS. Punica granatum (pomegranate) extract is active against dental plaque. J of Herbal Pharmacotherapy; (2006). 6: 79–92.

21) Gil MI, Tomás-Barberán FA, Hess-Pierce B, Holcroft DM and Kader AAAntioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J Agric Food Chem .(2000). 48: 4581-4589.

22) Mirdehghan SH and Rahemi M. Seasonal changes of mineral nutrients and phenolics in pomegranate (Punica granatum L.) fruit. Sci Hort (2007). 111: 120-127.

23) Mohammad T, Azar H , Karim D , Hamid M and Arezoo RProtective Effect of Pomegranate Seed Oil against Mercuric Chloride-Induced Hepatotoxicity in rat. Acta Poloniae Pharmaceutica - Drug Research, .(2016). 73 (4): 991-997

24) Adnaik RS, Gavarkar PS and Mohite SK: Evaluation of Antioxidant Effect of *Citrullus Vulgaris* against Cadmium-Induced Neurotoxicity in Mice Brain. Int J Pharm Sci Res 2015; 6(10): 4316-23..

25) Feldman, B. F., Zinkl, J. G., Jain, V. C. In Schalm's Veterinary hematology. 5th. ed. Lippincott Williams & Wilkins, Canada, (2000). P. 1145-1146 .

26) Thrall M. A., Campbell T. W., De Nicola D., et al. Veterinary Hematology and Clinical Chemistry. Lippincott Williams & Wilkins., A walters Kluwer Company., Philadelphia, Baltemore, New York, London. (2004). Pp.6-130.

27) Elbandy M.A., Ashoush I.S.Phytochemicals in pomegranate seeds and their effect as hypolipidemic agent in hypercholesterolemic rats, World Journal of Dairy & Food Sciences (2012) 7 (1), 85-92

28) Gutyj B., Binkevych V., Binkevych O. Hematological changes of rats after cadmium toxicsis. Haykobnn Bichnk Imehi .(2016) p.1(65).

29) Deepti G , Shabad P and K. K. Dua. Protective influence of dietary nutrients on antioxidant defense system in the blood of rats treated with cadmium. Advances in Applied Science Research, (2011). 2 (2): 69-78.

30) Flora, G.; Gupta, D.; Tiwari, A. Toxicity of lead: A review with recent updates. Interdiscip. Toxicol., (2012) . 5, 47–58.

31) El-Boshy, M.; Ashshi, A.; Gaith, M.; et al. Studies on the protective effect of the artichoke (Cynara scolymus) leaf extract against cadmium toxicity-induced oxidative stress, hepatorenal damage, and immunosuppressive and hematological disorders in rats. Environ. Sci. Pollut. Res., (2017) . 24, 12372–12383.

32) Milena A n, Aleksandra B D, Evica A. et al . Toxic Effect of Acute Cadmium and Lead Exposure in Rat Blood, Liver, and Kidney. Int. J. Environ. Res. Public Health 2019, 16, 274; doi:10.3390.

33) Borikov O. U., Kamilan P. A. The influence of cadmium chlorides and hydrogen peroxide on oxidation and fractional compound of lipids hepatocytes of rats // Ukrainian biochemical magazine. , (2004) . P.107–111.

34) James, R. and Sampath, K. Effect of the ion-exchanging agent, Zeolite, on reduction of cadmium toxicity: an experimental study on growth and elemental uptake in Heteropneustes fossilis (Bloch). J. Aquacul. Trop., (1999). 14: 65-74.

35) Kundu S, Sengupta S, Chatterjee S, Mitra, S, Bhattacharyya A. Cadmium induces lung

inflammation independent of lung cell proliferation: a molecular approach. J of , inflammation (2009) . 12: 6-19.

36) Ahmed HH, Abd-El-Dayem SM, Metwally FM, Foad NM .. Biological significance of grape seed extract against lung injury induced by formaldehyde inhalation in rats. Der Pharma Chemica (2015) 7: 64-78.

37) Oberdörster G Pulmonary deposition, clearance and effects of inhaled soluble and insoluble cadmium compounds. IARC Sci Publ. 1992;(118):189-204..

A Biometric Assessment of a Combined Topical Levofloxacin, Retinol, Cloxacillin and Ascorbic acid Against Facial Acne

Naser A Naser[1], Hussein Abdulkadhim A[2], Sahar A. Alhar[3], Karrar K. Alhaidary[4], Rana Talib Al-Nafakh[4]

[1]Assist. Prof. Dr. Department of Chemistry, Faculty of Science, [2]Assist Prof. Dr. Department of Pharmacology, Faculty of Medicine, [3]Dr. Lecturer. Department of Pharmacology, Faculty of Medicine, [5]Assist Lecturer. Department of Pharmacology, Faculty of Medicine, University of Kufa, P.O. Box (21), Najaf Governorate, Iraq.

Abstract

Acne vulgaris is a common pilosebaceous lesion affects skin over the face and upper chest. It has about 14 million incidence rate that cost 100$ for each case. In this study, biometric outcomes of the combined retinol, levofloxacin, cloxacillin, ascorbic acid and the hydrocarbon base vehicle on different types of acne lesions in human were investigated. Twenty one person were included in two groups; test (N =10) and control (N = 11) with follow up of objective software based biometric analysis parameters including: keratolysis induction, redness reduction and healing of infected lesion. The test formula revealed a significant keratolysis induction as compared with control. Eight out of 10 individuals with the test formula had keratolysis in comparison with 2 out of 11 had no keratolysis in controlled group, P-Value = 0.005. Similar effects were obtained in redness reduction (redness reduction ratio induced with test formula = 2.5 with confidence interval CI over 0.95) and impetiginization healing at $P < 0.05$. From the overall results, the combined retinol, levofloxacin, cloxacillin, ascorbic acid and the hydrocarbon base vehicle showed significant improvement in biometric outcomes of facial acne lesions.

Key words: *Facial acne vulgaris, RGB image processing, keratolysis, impetiginization*

Introduction

Acne vulgaris is a common dermatological lesion characterized by progressive popular to nodular skin lesion over the face and sometimes upper chest, back and shoulders [(1,2)]. Studies estimated 14 million presentations to the clinic suffering from acne vulgaris with 85% of cases were between 15-17 years of age[(3-5)] . It is presented in different forms: close, open, and black and white. It affects both sexes with average age incidence[(6)]. Acne vulgaris lesions cost 100$ for each case in average[(7)].

In Iraq and nearby countries prevalence of acne was 13.1% among skin diseases[(8,9)]. Acne vulgaris has multifactorioal causation. Androgen hyperactivity especially dihydrotestesterone[(10,11)] expression of binding factor (binding protein II and proline rich protein I) to Propionibacterium acne, excess inflammatory response to P. acne [(12)]and microcomidos formation with closure of sebaceous duct and accumulation of sebum [(13,14)]. Different cytokines and chemotaxis factors are noticed in excessive amount in acne lesion like IL1, IL12, IL8 and prostaglandins [(15)].Of the most common complications that are associated with this dermatological disease, facial and neck impetiginization and scar.

Factors that predispose to acne complications include hormonal hypersensitivity, hormonal imbalance, bacterial infections, age, weight, cosmetics and skin histopathological typing [(16,17)]. The most commonly used antiacne treatments include keratolytics, antibacterial and peeling agents [(18-20)]. However, a fraction of acne lesions are refractory to treatment that mandate more therapeutic researches.

The objective of this study is that to assess biometric outcomes of the combined topical formula of;

Corresponding author:
Hussein Abdulkadhim A
Department of Pharmacology, Faculty of Medicine, University of Kufa, P.O. Box (21), Najaf Governorate, Iraq.

levofloxacin, disintegrating agent, retinol, hydrocarbon mineral carboxylate, cloxacillin, pH buffering agent, ascorbic acid and vehicle base on different types of acne lesions in human.

Samples, Materials and Method

Study design

Controlled clinical trials of acne vulgaris was designed to exclude:

- Age: 25yr < age < 13yr
- Medications and antiacne intake
- Chronic medical illnesses like HT,DM and hormonal disturbances
- Weight: 70 kg < wt < 50 kg

Prerequisites of medical ethics (according to Geneva and Helsinki declarations) submitted to local ethical committee.

- All of the used drugs and excipients are FDA approved for safety and efficacy
- All of the used drugs were passing phase I (i.e. not used as a first time)
- Benefit is prevailed
- All individuals were informed about study design and expected side effects
- All individuals permission, autonomy and consents have been obtained
- All of the used drugs were through topical route of administration
- All individuals were prior tested for any skin hypersensitivity and side effects.
- All individuals names, private secrets, faces were respected and insured
- All rights were reserved.
- All were free to be subjected to medical treatment or test treatment.

Material

1) **Hydrocarbon cream o/w**

Of multiple esters; Myrsticate, glycol monostearate, Na palmitate, Na stearate, isopropyl alcohol and water 5.05 ml

2) **Active ingredient and vehicle base:**

- Retinol (Egypt, product date and expiry date; PD-ED: 2013-2015)

Package of 200000 IU. The used dose in designing the topical test formula against acne was 0.05%.

- Cloxacillin (Ajanta, India, PD-ED: 2014-2015). The used dose was o.1%
- Levofloxacin (Pharma International, Jordan; PD-ED: 2014-2015). The used dose was 0.1%.
- Ascorbic acid (Merck, PD-ED: 2013-2015). The used dose was 0.1%
- Hydrocarbon base was used as a vehicle of Myrsticate, glycol monostearate, Na palmitate, Na stearate, isopropyl alcohol and water 5.0 mL.

Constituents of the combined antiacne formula;

(Levofloxacin, disintegrating agent, retinol, hydrocarbon mineral acyls, cloxacillin, pH buffering agent, ascorbic acid, vehicle base)

Constituents of the blank controlled formula;

(Hydrocarbon base+ mineral oils+ pH buffer, disintegrating agent)

Pharmaceutical analysis is done to assess formula pH and physical consistency was assessed with PHELECT computerized pH meter electrode (USA). pH was buffered around 6.3.

Methods

Acne sample selection

The lesion to be monitored with biometric method was not randomly selected

Criteria of selection

- More prominent lesion in the face is selected to be monitored.
- Type of lesion was randomized (to include all presenting lesion types for further analysis and concluding the overall effect on all types)

- Monitoring was done in conserved temperature but different ambient lighting because this was processed and normalized by software.

- Selection of blank treated and test treated was randomized

- There was no person with any application of either test or blank to insure ethical requisites. On the other hand, no person was with standard medical treatment.

Methods of observation and analysis

Places of application were therapeutic research lab/ Kufa College of medicine for assessment of students.

Private lab for assessment of the volunteers out of the college as well.

Ambient conditioning

Lighting was objectively evaluated to be controlled

Ambient temperature has averaged 22-25 ºC

A prior imaging of the lesion was taken then after 4 day of the treatment another image was acquired for MIP.

MIP was a mathwork 2013a image processing blockset design by Dr Hussein AbdulKadhim for processing and analyzing RGB and lesion pattern for:

1- RGB shift

2- Lesion dimension

3- Pattern of lesion response (keratolysis, impetiginization, dimentions and bevel).

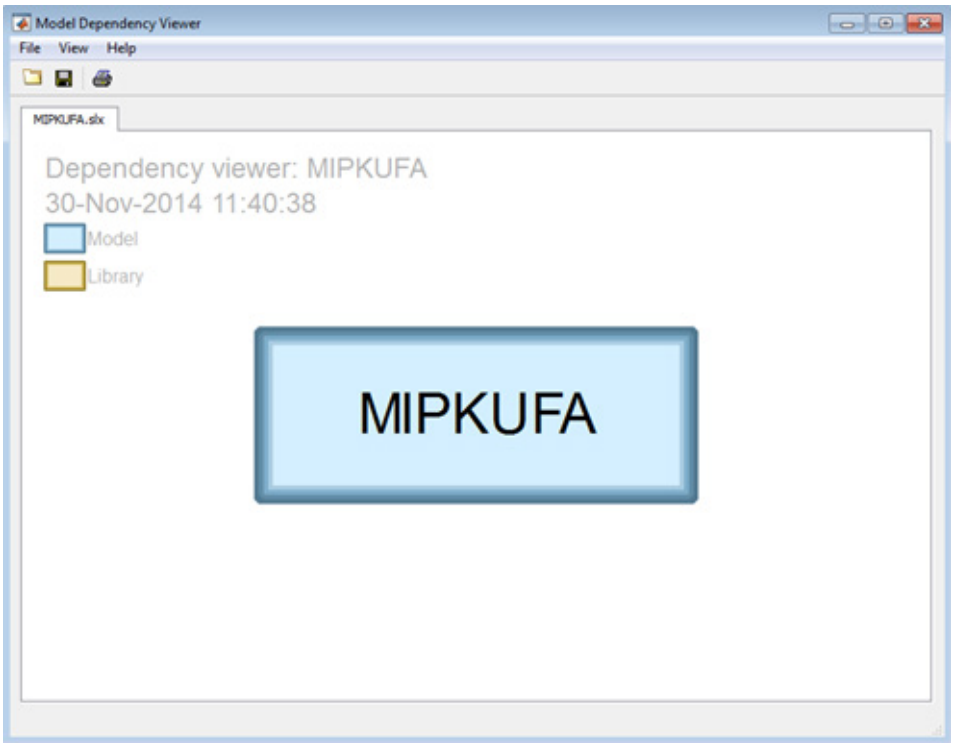


Figure 1: Mathwork 2013a image processing and analysis used for analysis of the outcomes by the combined Microsoft thermograph camera.

Treatment Mode

The entire face was messaged with the test combination and left overnight then morning washing. Another application was done for 1hr duration to be washed prior to attending time.

Biometric Analysis Method

The biometric set composed of a Microsoft combined thermographic tissue camera with MIP for objective image analysis.

Analyzing histogram vector RGB and lesion in form

of red pixelate shift was objectively monitored and data were analyzed statistically with spline interpolation and risk reduction at 0.95 C.I. Discrete data was analyzed with chi square test at $P < 0.05$. Statistical software packages were Matlab 2013 statistical toolbox and Minitab 2014 statistics.

Results

1- **Findings and analysis of keratolytic activity of the test combined topical formula.**

Chi-Square Test: test; control

Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

	test	control	Total
1	8	2	10
	4.76	5.24	
	2.202	2.002	
2	2	9	11
	5.24	5.76	
	2.002	1.820	
Total	10	11	21

Chi-Sq = 8.025; DF = 1; P-Value = 0.005
1 cells with expected counts less than 5.

Figure 2: Number of individuals who showed keratolysis in response to the applied test formula as compared to those used a blank cream base after 14 days of treatment. So that 8 out of 10 individuals with the test formula had keratolysis in comparison with 2 out of 11 had no keratolysis in controlled group. P-Value = 0.005.

2- **RGB analysis findings for acne lesions**

Color model interpolation and the mean red value estimation with redness reduction ratio.

In control group:

MIP red value was reduced from 240-200 = 40

In test group:

MIP red value was reduced from 250-150 = 100

So the redness reduction ratio of test formula = 2.5 at C.I. 0.95.

Figure 3: Mean values of red bins by which the MIP had shifted after 14 days of treatment. This was a direct indicator of acne reactivity in response to treatment.

3- Lesion impetiginization response

Chi-Square Test: test; control

Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

	test	control	Total
1	1	5	6
	3.25	2.75	
	1.558	1.841	
2	12	6	18
	9.75	8.25	
	0.519	0.614	
Total	13	11	24

Chi-Sq = 4.531; DF = 1; P-Value = 0.033
2 cells with expected counts less than 5.

Discussion

A trial of assessment of topical antiacne formula has a significant consideration since it can be reasonably safe alternate to systemic administration of drugs for prolonged period.

The reason behind selecting combined active ingredients in designing the test formula was to induce synergism since P. acne, S. aureus and S. pyogen are rapidly emerging resistance against the commonly used antimicrobials[21,22] and to potentiate peeling with retinol and keratolysis with ascorbic acid[23,24]. This principle causes augmentation of antiacne effect.

However this study concerned a limited number of population and needs for further confirmation in larger samples. Overall clinical and biometric outcomes are best to be included in further studies.

The clinical evaluation of keratolytic activity of the combined formula showed highly significant induced keratolysis (Chi-Sq = 8.025; DF = 1; P-Value = 0.005) in comparison with blank treated group. That was a clinical sign of improvement since keratlysis can convert closed comedos to opened type. Moreover, keratolysis insure more antiacne drug absorption fraction since it causes thinning of the corneocytes portioning. Different studies showed the importance of the use of keratolytics in treatment of acne[25].

Redness is a major indicator of inflammation. It could be assessed objectively by RGB shift analysis. Redness reduction ratio was 2.5 at C.I. 0.95.

Test formula revealed a significant remission of impetiginization in comparison with blank treatment (Chi-Sq = 4.531; DF = 1; P-Value = 0.033).

That effect may be attributed to the synergistic activity of levofloxacin and cloxacillin. Some studies on assessment of comedolytic effects of ciprofloxacin and ampicillin revealed significant influence of these drugs on improving acne[26-29].

Conclusion

From the overall results, the combined retinol, levofloxacin, cloxacillin, ascorbic acid and the hydrocarbon base vehicle showed significant improvement in biometric outcomes of facial acne lesions. Larger sample size is necessary for further confirmation of the antiacne activity of the test formula. We recommend that other congeners of the used antimicrobials and keratolytics are to be included. And, for future studies, other comparative studies between antiacne drugs alone and in combination to determine the synergistic ratio.

Acknowledgement: A special gratitude and thanks is to the head of department of pharmacology-College of Medicine at University of Kufa for his help to use the lab. Thanks is to the technician in the department of pharmacology for his help in conducting the topical formulation.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1- Strauss JS, Krowchuk DP, Leyden JJ, Lucky AW, Shalita AR, Sigfried EC. Guidelines of care for acne vulgaris management. J Am Acad Dermatol. 2007; 56(4): 651-63.

2- Zaenglein A, Pathy A, Schlosser B, Alikhan A, Baldwin H, Berson D. Guidelines of care for the management of acne vulgaris. J Am Acad Dermatol. 2016; 74(5): 945-973.e33.

3- Anthony J. Incidence, prevalence and pathophysiology of acne. Adv. Stud.Med.2008; 8(4):100-105.

4- Friedlander SF, Eichenfield LF, Fowler JF, Fried RG, Levy ML, Webster GF. Acne epidemiology and pathophysiology. Semin Cutan Med Surg. 2010; 29(21): 2-4.

5- Bhate K, Williams HC. Epidemiology of acne vulgaris. British Association of Dermatologists. 2013;168, 474–485.

6- Soodabeh Z; Behrouz V; Hamid A. Determination of microbial agents of acne vulgaris and Propionibacterium acnes antibiotic resistance in patients referred to dermatology clinics in Kerman, Iran. Jundishapur Journal of Microbiology. 2011; 4(1): 17-22.

7- David J. Leffel. The Acne Family. 2000; 15(8): 329-341.

8- Bilgili M, Yildiz H, Sarici G. Prevalence of skin diseases in a dermatology outpatient clinic in Turkey. A cross-sectional, retrospective study. Journal of Dermatol Case Report. 2013; 7(4):108-120.

9- Asude K. Polat, Yavuz Yesilova, Emine T. Alatas, Asli A. Belli, Gursoy Dogan, Metin Picakciefe. Prevalence of skin diseases of the pediatric population in the Southeastern Anatolia, Turkey. Medicine Science 2018; 7(3): 664-667.

10- Lai J, Chang P, Lai K, Chen L, Chang C. The role of androgen and androgen receptor in skin-related disorders. Arch Dermatol Res. 2012 ; 304(7): 499-510.

11- Peter T. Riis, Hans C. Ring, Lotte Themstrup, Gregor B. Jemec. The Role of Androgens and Estrogens in Hidradenitis Suppurativa – A Systematic Review. Acta Dermatovenerol Croat. 2016 ; 24(4): 239-249.

12- Perry D, Alexandra R; Lambert P. Propionibacterium acnes: Infection beyond the skin. Expert Review of Anti-infective Therapy. 2011; 9(12): 1149-56.

13- Chandersekar L. Review on Pathophysiology and Treatment of Acne. RJPBCS. 2013; 4(2): 1355-1361.

14- Shyam BP. Review on Pathophysiology and Treatment. Asian J Pharm Clin Res. 2016; 9(4): 54-59.

15- Tanghetti EA. The Role of Inflammation in the Pathology of Acne. The Journal of Clinincal and Asthetic Dermatology. 2013;6(9):27-35.

16- Tahir M. Efficacy and adverse effects of systemic isotretinoin therapy. Journal of Pakistan Association of Dermatologists. 2011; 21: 38-42.

17- Vallerand IA, Lewinson RT, Farris MS, Sibley CD, Ramien ML, Bulloch AG. Patten SB. Efficacy and adverse events of oral isotretinoin for acne: a systematic review. Br J Dermatol. 2018;178(1):76-85.

18- Titus S, Hodge J. Diagnosis and Treatment of Acne. American Family Physician. 2012; 86(8):734-740.

19- Ivan S, Galstyan GR, Solomatina KY, Bondarenko ON, Mitish VA, Doronina LP. Critical limb ischemia and diabetic foot treatment in a multidisciplinary team setting. Cardiac & Vascular Update. 2012; 2(11): 24-29.

20- Becker, M., Wild T. Zouboulis C. Objective Assessment of Acne. Clinical Dermatology. 2017; 35(2): 147-155.

21- Malahlela P, Motswaledi M. Management of mild to moderate acne vulgaris. South African Family Practice. 2013; 55(3): 241-244.

22- David CH, David GA. The Multidisciplinary Team Approach To The Diabetic Foot. *Podiatry Today*. 2016; 29(6): 16-21.

23- Sartorius K, Boer J, Gregor B, Jemec J. Topical Treatment. Hidradenitis Suppurativa. 2006; 20(3): 150-160 .

24- Linda S, Henry F. Topical Treatments in Acne Vulgaris: Guidance for the Busy Dermatologist. J Drugs Dermatol. 2015; 14(6): 567-572.

25- Decker A, Graber EM. Over-the-counter Acne Treatments: A Review. J Clin Aesthet Dermatol. 2012; 5(5): 32-40.

26- Webster GF, Graber EM. Antibiotic Treatment for Acne Vulgaris. Semin Cutan Med Surg. 2008; 27(3):183-187.

27- Caitlin WH, Joseph KC, Hikmet K, Nestoras M, Ronald LS, James HB, Christopher JA. The Society for Vascular Surgery Wound, Ischemia, and foot Infection (WIfI) classification system correlates with cost of care for diabetic foot ulcers treated in a multidisciplinary setting. J of Vascular Surgery. 2018; 67(5): 1455–1462.

28- Vali A, Faghihi G, Zaghian N, Koosha M. The Efficacy of Topical Solution of 0.3% Ciprofloxacin in Treatment of Mild to Moderate Acne Vulgaris. Iranian Red Crescent Medical Journal. 2009; 11(1): 23-27.

29- Sapkota A, Coker M, Rosenberg Goldstein R, Atkinson N, Sweet S, Sopeju P et al. Self-medication with antibiotics for the treatment of menstrual symptoms in southwest Nigeria: a cross-sectional study. BMC Public Health. 2010; 10(1): 23-29.

Assessment the Efficacy of Arthrocentesis with Corticosteroid and Arthrocentesis with Sodium Hyaluronate in Treatment Temporomandibular Joint Disorders: A Comparative Study

Samer Majeed Mohammed[1], Muntathar Muhsen Hassan Abusanna[2], Zina Ali Daily[3]

[1]University of Al-Ameed/College of dentistry-Iraq

Abstract

Background: Temporomandibular joint disorders (TMD) refer to a group of heterogeneous pain and dysfunction conditions involving the masticatory system, reducing life quality of the sufferers. **Aim of study:** The aim of the study was to evaluate the effectiveness of arthrocentesis with corticosteroid (betamethasone) injection and with sodium hyaluronate injection in treatment temporomandibular joint disorders, in conjunction with a stabilizing splint, for improving function and reducing pain, for preventing further deterioration of the TMJ dysfunction, to maintain improvements over time, and compare between them. **Patients and methods:** Fifty –four patients with TMJ disorders with age limit between 18 and 55 years of age, 9 males and 36 females, were enrolled in this study. Patients were randomly divided into two groups, in which one group received arthrocentesis with intra-articular corticosteroid (betamethasone) injection (1 ml), and another group received arthrocentesis with intra-articular sodium hyaluronate injection (1 ml) in superior joint space ,in single puncture. Patients were followed at regular interval of 7th day, one month, 3, 6, 9, 12 months (follow up) after last injection. **Results:** The mean age of patients was 8.873 ± 34.112 years in Group-A treat by betamethasone and10.973± 33.27 years in Group-A treat by sodium hyaluronate (ranged from 18.2 to 55.0 years).

Keywords: *Arthrocentesis, corticosteroid(betamethasone),Sodium hyaluronate, temporomandibular joint disorders*

Introduction

Internal derangement of the temporomandibular joint (TMJ) is a progressive disorder which usually starts with clicking associated with normal mouth opening (anterior disc displacement with reduction),to a stage where clicking gradually ceases but restricted mouth opening ensues (closed lock). This was attributed to a nonreducible anteriorly displaced articular disc acting as an obstacle to the gliding condyle[1] . In the meantime, the pathological changes were found in synovial membrane and synovial fluid. Hyaluronic acid is a principal component of the synovial fluid which plays an important role in nutrition, lubrication, anti-inflammation and cartilage protection and repairing. The synthesis, molecule weight, and concentration of hyaluronic acid are decreased during TMD and cause TMJ degenerative changes[2].TMD treatment can be divided into two categories: conservative method and surgical method. Among the surgical interventions, arthrocentesis is generally suggested for patients who are not responsive to conservative therapy[3].

Arthrocentesis is generally suggested for patients who are not responsive to conservative therapy[4]. Arthrocentesis is an easy, minimally invasive, highly efficient procedure designed to decrease joint pain and increase the range of mouth opening in patients with closed lock of TMJ[5].This improvement in clinical outcomes after arthrocentesis can be attributed to the facts that the flow of liquid under pressure in joint causes flushing of catabolites, distension of joint with breakage of adhesions, and mobilization of disc[6].

Corticosteroids (CSs) are anti-inflammatory drugs that interrupt the inflammatory and immune pathways. They have been used for both therapeutic and diagnostic purposes. Also, they showed their palleviating effects by suppressing inflammatory responses[7] .Intra-articular corticosteroid injection alone or after arthrocentesis provides long-term palliative effects on subjective symptoms and clinical signs of TMJ pain[8].

Hyaluronic acid (HA) is a polysaccharide which is produced by chondrocytes and synoviocytes of the joints. HA has been shown to improve and restore normal lubrication in joint, provide nutrition to the avascular articulating disc, and stabilize the joint. The therapeutic mechanism of action of HA is chondroprotection, effect on proteoglycan and glycosaminoglycan synthesis, anti-inflammatory, mechanical (viscosupplementation), effect on subchondral bone, and analgesic[2,9].

HA is a polysaccharide of low, medium, or high molecular weight ,its properties can vary in relation to its molecular weight and shape, and has been used successfully as a TMJ injection, to reduce inflammation , restore normal lubrication and cartilage repair[2].

These have motivated us to perform the current study , to evaluate the effectiveness of arthrocentesis with corticosteroid (betamethasone) injection and with sodium hyaluronate (low molecular weight) injection in treatment temporomandibular joint disorders, in conjunction with a stabilizing splint, for improving function and reducing pain, for preventing further deterioration of the TMJ dysfunction, to maintain improvements over time, and compare between them during 12 months following injection.

Patients and Method

Fifty –four patients with TMJ disorders with age limit between 18 and 55 years of age, 9 males and 36 females, were enrolled in this study. All patients were examined clinically and radiolographically. Based on the history and examination of patient a diagnosis of internal derangement was made. Patients were informed about the procedure, it is possible complication and about the material used and after the consent, patients were randomly divided into two groups, (27 in each group) and arthrocentesis was performed in each group following which 1 ml of betamethasone was given in first group and 1 ml of sodium hyaluronate in second group in superior joint space. Patients were followed at regular interval of 7th day, one month,3,6,9,12 months (follow up) after single injection. Assessment of clinical outcome was done in terms of reduction in pain (visual analog scale score), maximum mouth opening (MMO) in millimeters, painful/pain-free lateral or protrusive jaw movement, and clicking/crepitus of joint in pre-treatment visit about 1week before injection and post-treatment follows up visits . This study was performed in Samir Dental Clinics in Karbala city, from October 2016 to Jun 2019. All patients were diagnosed with TMD based on Clinical finding supported by CT scan. In this study , selection of patients based on the following

Inclusion Criteria :

1- Clinical diagnosis of anterior disc displacement(Limitation of mouth opening, Pre-auricular pain, temporal and occipital tenderness, headache,Persistence of symptoms at least for 3 months, Clicking).

2-CT scan(soft tissue window depend on disk density) diagnosis of anterior disc displacement with reduction.

Each patient received pharmacotherapy and then if no or delay response splint fabricated then if no progression in treatment, arthrocentesis with intra-articular betamethasone injection and arthrocentesis with intra-articular sodium hyaluronate injection in superior joint space were done .Patients were informed of the use of their medical records. Ethical approval for the study was obtained from the ethical committee.

Also, we exclude other patients according to **exclusion criteria :** Systemic disease, Arthritis or history of condylar trauma, Degenerative change of condylar head,Facial asymmetry, retrognathism, prognathism. Fibromyalgia,use of NSAIDS within 48 hours,allergy to study medications,edentulous subjects pregnancy or breast feeding,

The statistical analysis was carried out using Statistical Package for Social Sciences (SPSS Inc). All quantitative variables were estimated using measures of central location (mean) and measures of dispersion (standard deviation). As data was normally distributed, paired t-test was applied for comparison of every two visits of each group. All statistical tests were two-sided and performed at a significance level of α=.05.

Results

The mean age of patients was 8.873±34.112 years in Group-A treat by betamethasone and10.973± 33.27 years in Group-A treat by sodium hyaluronate (ranged from 18.2 to 55.0 years). A detailed sex and age distribution is shown in (Table- 1).more reducing of mean and ±SD(standard deviation)values of the pain intensity, maximum mouth opening, joint click and deviation on opening were recorded in post-treat at 12 month follow-up visit of Group -B(SH)than pre-treat visit ,followed by Group-A(CS) is shown in (Table-2).

Inter study visits comparisons of each group regarding of the pain intensity, maximum mouth opening, deviation mouth opening and joint click revealed, Highly signifiant differences between pre-treatment visit and post-treat at 12 month follow-up visit after the single injection in both groups (Table-3) HS differences between post-treat at 12 month follow-up visit of Group -B(SH)and post-treat at 12 month follow-up visit of Group - A(CS) (Table -4).

Table 1: Sex and age distribution

Group	Age	Gender	
	mean±SD	male	Female
Group-A treat by betamethasone	8.873± 34.112	5	22
Group-A treat by sodium hyaluronate	10.973± 33.27	4	23

Table-2: Descriptive statistics of the pain intensity, maximum mouth opening, joint click and deviation on opening of mouth

Clinical Para meter	Group A Pre-treat mean±SD	Group-A Post-treat at 12 month mean±SD	Group-B Pre-treat mean±SD	Group-B Post-treat at 12 month mean±SD
Pain intensity	7.27±0.273	1.85±0.260	7.77±0.381	0.36±0.231
Maximum mouth opening	35.16±0.259	41.02±0.281	35.48±0.411	43.67±0.227
Joint click	8.41±0.440	1.60±0.247	8.79±0.352	0.45±0.404
Deviation of mouth	7.77±0.278	1.94±0.249	8.38±1.571	0.41±0.345

Table-3: Comparisons between pre-treatment visit and follow-up visits of each group in the pain intensity, maximum mouth opening, joint click and deviation on opening of mouth

Clinical Para meter	Group A Pre-treat vs. Post-treat at 12 month			Group B Pre-treat vs. Post-treat at 12 month		
	T-test value	Df	P-value	T-test value	Df	P-value
Pain intensity	84.043	26	0.000	90.292	26	0.000
Maximum mouth opening	80.995	26	0.000	112.061	26	0.000
Joint click	62.479	26	0.000	95.923	26	0.000
Deviation of mouth	24.963	26	0.000	88.499	26	0.000

***Df: degree of freedom**

Table -4: comparisons between post-treat at 12 month follow-up visits of each group in the pain intensity, maximum mouth opening, joint click and deviation on opening of mouth

Clinical Para meter	Group-A Post-treat at 12 month vs. Group-B Post-treat at 12 month		
	T-test value	Df	P-value
Pain intensity	21.871	52	0.000
Maximum mouth opening	37.257	52	0.000
Joint click	12.291	52	0.000
Deviation of mouth	18.209	52	0.000

Discussion

In the present study, clinical parameters such as pain, MMO, lateral and protrusive movement of jaws, and improved significantly in both the treatment arthrocentesis with intra-articular injection corticosteroid and arthrocentesis with Sodium hyaluronate (SH). However, more significant improvement in pain, MMO, lateral and protrusive movement was observed in patients receiving arthrocentesis with intra-articular SH injection .

The outcome of the recent study is agree with systematic review of Eduardo etal.2013[10], they were found that the effects of intra-articular injections with sodium hyaluronate are similar to those regarding the injections with corticosteroids to control TMJ internal derangements at short and medium terms , while in long-term treatments, injections with sodium hyaluronate showed better results.

The result of recent study is agree with study of Kapusuz G.et al. 2014[11] ,that studied effectiveness intra-articular injections of hyaluronic acid, tenoxicam and betamethasone on the relief of temporomandibular joint disorder complaints , they found that HA produced better pain relief scores when compared to the other anti-inflammatory agents studied.

Radiological assessment preoperatively and postoperative 12 months follow-up with CBCT was showed significant difference. The erosion on condyles disappeared in the patients in both groups, it was significant, and showed radiological thin layer of new bone formation, remodeling of condyles and glenoid fossa in patients who treated by arthrocentesis with betamethasone ,and cortical bone formation and remodeling of severe degenerative changes at 12months follow-up in patients who treated by arthrocentesis with sodium hyaluronate, it is highly significant in patients receiving arthrocentesis with sodium hyaluronate injection than significantly in patients receiving arthrocentesis with betamethasone.

The result of recent study is agree with study of Li et al. 2015[12] ,that studied changes of TMJ disorders in CBCT in patients who received HA injection in superior joint and reported cortical bone formation and remodeling of severe degenerative changes by 9 months follow-up.

This improvement in clinical outcomes after arthrocentesis can be attributed to the providing viscosupplementation to joints, HA has anti-inflammatory effects on inflammatory mediators , and protection against the disintegration of proteoglycans and cytotoxity induced by oxygen free radicals. IL-1β is the key mediator in anti-inflammatory effects of HA and is regulated through HA-CD44 binding. IL-1β suppression results in downregulation of matrix metalloproteinases which also aids in anti-inflammatory effects of HA and further suppression of pro-inflammatory mediators IL-8, IL-6, prostaglandin E2, and TNF-α provides anti-inflammatory effects of intra-articular HA treatment[6]. In addition, it affects leukocyte adhesion, proliferation, migration and phagocytosis ; it directly influences the control mechanism of monocyte activation; in the cartilage it has been seen to suppress degradation of the cartilaginous matrix by fibronectin fragments[9,13,14].

Hyaluronic acid is found in the extracellular matrix of several connective tissues of high molecular weight, including joint cartilage and synovial fluid .In such

sites, HA molecules are predominantly synthesized ,It is synthesized by synoviocytes, fibroblasts and chondrocytes present in the connective tissue, In addition, it is the largest natural component of SF and an important component of the articular cartilage. Moreover, activates intrinsic repair processes of the cartilage and normalizes the endogenous production of HA by the synoviocytes, stabilize the extracellular matrix, stimulate the proliferation of chondrocytes, regulate the production/degradation of type II collagen,and metabolic HA activity in cell renewal helps the nutrition of avascular zones of the disk and joint cartilage through its combination with glycosaminoglycans coming from proteoglycans produced by chondrocytes[9,14].

It is the major component of the synovial fluid and has an important role in lubrication, Its action stems from the ability of the polysaccharides to connect to each other when they are in solution, forming a network that provides a high degree of viscosity to the SF so that it reduce joint friction coefficient ,that is the main risk factor for degenerative joint pathologies , maintaining intra-articular homeostasis by promote a better distribution of forces and load absorption of articular tissues[6,10].In cases of inflammatory and degenerative changes of joints, the concentration and molecular weight of HA acid reduced, theraputical effect sodium hyaluronate increases the concentration and molecular weight of HA in the synovial fluid,restoring tissues lubrication and nutrition as well as minimizing mechanic stress[9].Moreover, intra-articular SH injection is avoided sensitization of pain receptors in joints disorders ,by modulating neurotransmission and vasodilatation processes, provides an analgesic effect by blocking receptors and endogenous substances that cause pain in synovial tissues and. In addition, it promotes a release of adhesion areas between the articular disc and the mandibular fossa, increasing joint mobility and allowing better synovial fluid circulation[6,9,10].

Corticosteroids have a potent anti- inflammatory effect on synovial tissue and are known to reduce effusion, decrease pain and bring about an increase in range of motion of synovial joints[15].

Glucocorticoids have a very original mechanism of action, essentially genomic (transcriptional) and characterized by the activation (transactivation) or inhibition (transrepression) of numerous target genes. These molecules act in many cells, including not only innate immunity cells (macrophages, granulocytes, mast cells) and adaptive immunity cells (lymphocytes), but also other cells (fibroblasts, epithelial and endothelial cells)[10].

Therefore arthrocentesis with sodium hyaluronate injection is more effectiveness than arthrocentesis with betamethasone injection in therapeutic and return TMJ of healthy status in long term palliative effects .

Conclusion

In this study, the technique of arthrocentesis using 0.9% normal saline solution with betamethasone injection, with occlusal splint in group A and arthrocentesis with intra-articular injection of sodium hyaluronate in superior joint space with occlusal splint wear in group B , where showed therapeutic benefits , simplicity , safety , patients acceptance of injection technique and lack of significant side effects and complication. Both techniques increased maximal mouth opening, lateral movements, and function, while reducing TMJ pain and noise. Although patients benefitted from both techniques, arthrocentesis with injection of SH is significantly superior to arthrocentesis with betamethasone injection .As well as, Radiological finding is showed highly significant in patients receiving arthrocentesis with HA injection than significantly in patients receiving arthrocentesis with corticosteroid .

Refreneses

1- Albright JA, Brand RA. The scientific basis of orthopedics. 2nd ed. Norwalk(CT): Appleton and Lange; 1987. p. 373-86.

2- Long X. Zhonghua Kou Qiang Yi Xue Za Zhi. Review article,Intra-articular injections of hyaluronic acid for anterior disc displacement of temporomandibular joint. 2017. PMID 28279053 [Indexed for MEDLINE]]

3- Yustin D, Neff P, Rieger MR, Hurst T. Characterization of 86 bruxing patients with long-term study of their management with occlusal devices and other forms of therapy. J Orofac Pain 1993;7: 54-60.

4- Nitzan DW: Arthrocentesis for management of sever closed lock of the temporomandibular joint: current controversies in surgery for internal derangement of the temporomandibular joint, Atlas Oral Maxillofac Surg Clin North Am,1994; 6:245.

5- Sato S, Sakamoto M, Kawamura H, Motegi K. Disc position and morphology in patients with

nonreducing disk displacement treated by injection of sodium hyaluronate . Int J Oral Maxillofac Surg 1999;28:253.

6- Tikaram Gurung, R. K. Singh, Shadab Mohammad, U. S. Pal, Abbas Ali Mahdi, and Manoj Kumar. Efficacy of arthrocentesis versus arthrocentesis with sodium hyaluronic acid in temporomandibular joint osteoarthritis: A comparison.Natl J Maxillofac Surg. 2017 Jan-Jun; 8(1): 41–49. 2017PMC5512408

7- Comert Kilic S. Does Injection of Corticosteroid After Arthrocentesis Improve Outcomes of Temporomandibular Joint Osteoarthritis? A Randomized Clinical Trial. J Oral Maxillofac Surg. 2016;74:2151–8. [PubMed] [Google Scholar]

8- Barkin S, Weinberg S. Internal derangements of the temporomandibular joint: the role of arthroscopic surgery and arthrocentesis. J Can Dent Assoc 2000;66:199-203.

9- Veronica Iturriaga; Bélgica Vásquez; Carlos Manterola, & Mariano del Sol.Role of Hyaluronic Acid in the Homeostasis and Therapeutics of Temporomandibular Joint Osteoarthritis .Int. J. Morphol. 2017;35(3):870-876,.

10- Eduardo MachadoI; Daniel BonottoII; Paulo Afonso CunaliI.ORIGINAL ARTICLE, Intra-articular injections with corticosteroids and sodium hyaluronate for treating temporomandibular joint disorders: a systematic review.Dental Press J. Orthod. Maringá Sept./Oct. 2013 ; vol.18: no.5 .

11- Kapusuz Gencera, MahmutÖzkirişa Aylin Okurb, MuratKorkmazc, LeventSaydam. Acomparative study on the impact of intra-articular injections of hyaluronic acid, tenoxicam and betametazon on the relief of temporomandibular joint disorder complaints..Journal of Cranio-Maxillofacial Surgery. October 2014; 42(7): 1117-1121.

12- Li C, Long X, Deng M, Li J, Cai H, Meng Q. Osteoarthritic changes after superior and inferior joint space injection of hyaluronic acid for the treatment of temporomandibular joint osteoarthritis with anterior disc displacement without reduction: A cone-beam computed tomographic evaluation. J Oral Maxillofac Surg. 2015;73:232–44. [PubMed]

13- Sara Bergstrand, Hanne K Ingstad), Anne Møystad, and Tore Bjørnland.Long-term effectiveness of arthrocentesis with and without hyaluronic acid injection for treatment of temporomandibular joint osteoarthritis. Journal of Oral Science, Original,J-STAGE Advance Publication: February 28, 2019

14- Eduardo GrossmannI; Eduardo JanuzziII; Liogi Iwaki FilhoI. REVIEW ARTICLE ,The use of sodium hyaluronate in the treatment of temporomandibular joint disorders. Scielo Journal of Oral Science, Original. São Paulo Oct./Dec. 2013; vol.14: no.4

15- Koop S, Carlsson GE, Haraldson T, Wenneberg B. The short term effect of intra-articular injection of sodium hyaluronate and corticosteroid on temporomandibular joint pain and dysfunction. J Oral Maxillofac Surg. 1985;43:429–435. doi: 10.1016/S0278-2391(85)80050-1. [PubMed] [CrossRef] [Google Scholar]

Aqueous Extract of Date Palm Fruit (*Phoenix dactylifera*) Protect Liver Against Cyproterone Acetate Toxicity in Male Mice

Nuha Yaarub Al-Harbi[1], Eman Fadhel Abbas Awadh[1], Wasan Najim Abdul-Sada[2]

[1]University of Babylon, College of Science, Dept. of Biology, Iraq, [2]Basic Science Department, College of Dentistry, University of Babylon, Hillah, Iraq

Abstract

The cyprote.rone acetate (CPA) is an antiandrogen drug that is used in the treatment of prostate cancer, which is related to drug-induced liver injury (DILI). The aim of this study the effect of the water extract of one of the local dates on the side effects of using cyproterone acetate on liver tissue in white mice. Forty from albino mice male were divided into 8 equal groups received orally in one ml as follows. Group 1: distilled water passive control, group 2: corn oil positive control, group 3: received with 5 mg/kg body weight CPA , group 4: received, with 20 mg/kg CPA, group 5: received with 5 mg/kg CPA & 60 mg/kg date palm extract, group 6: received, with 5 mg/kg CPA & 120 mg/kg date palm extract, group 7: received , with 20 mg/kg CPA & 60 mg/kg date palm extract, group 8: received with 20 mg/kg CPA & 120 mg/kg date palm extract lasted for 21 days.

Showed a histological study of the liver remarkable degeneration of hepatocytes associated with interstitial necrosis and blood vessel congestion.

The current study proved that the water extract of dates has a weak effect in the repair of damage in the liver tissue to treatment for the low dose of cyproterone acetate only.

Keywords: *Cyproterone acetate, Date palm fruit, hepatotoxicity, histopathology.*

Introduction

CPA is synthetic progesterone and antiandrogenic component administered It is used to treat many physical conditions in pro.state cancer and also in breast cancer, serious acne, womanly hirsutism, precocious puberty, hypersex.uality [(1).]

Drugs can have direct toxic effects (dose-dependent) or elicit hypersensitivity or metabolic distinctive reactions (dose-independent) that can take place at any time during the course of therapy [(2).] Hepatotoxicity signs resulting from the use of both steroidal androgens and nonsteroidal antiandrogens causes many cases asimmunoallergic cytotoxic reactions, cholestasis, auto.immune hepatitis [(3 &4)], acute hepatitis, fulminant hepatic fail [(5)] cirrhosis[(1)]and ultimately, CPA has been imputed a hepatocellular mutagenic capacity leading to hepato-carcinogenesis[(7)]. Many treatments are for natural products can use because they are cheap and easy to obtain. The Prophet Mohammed (Peace Be upon Him) recommended the use of natural products as medicine for certain diseases[(8)]. In traditional medicine, herbal medicines are widely used around the world, where palm pollen grains are widely used as an anti-hepatotoxicity[(9&10)].

Pho.enix dac.tylifera L. plant is one of the elderly cultivated plants in the Middle East and North Africa. The fruits of dried dates contain 8 phenolic acids (gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, and ferulic acid [(11)&(12).] In addition, date fruits contain

Corresponding author:
Nuha Yaarub Al-Harbi
University of Babylon, College of Science, Dept. of Biology, Iraq,

Ascorbic acid, β-carotene, nicotinic acid, riboflavin and thiamine [13]. and include twenty-one free amino acids (leu.cine, α-alanine, and proline were predominant), and the amides aspargine and glutamine were particular in P. dactylifera [14]. Another study indicated that date palm fruit contains cholesterol, campesterol, stigmasterol, β-sitosterol, and fucosterol [15]. Every date types are a user provenance of natural antioxidants It can be considered effective food [16] since date fruit extract had a powerful antioxidant and antimutagenic specialty [17].

Vari.ous studies have that the date fruit extract has been shown to ameliorate liver damage in rat, inhibit swelling and tumors, suppress the growth of Streptococcus pyogenes and improve sperm parameters [18) &(19).

Materials and Method

Preparation of extract

Fresh date palm fruits (*Pho.enix dactylifera L., Palmae*) were provided from a local market in Hilla (Babil, Iraq), dried at room temperature, and were manually isolated from the pits. The flesh of the dried *P. dactylifera* fruits was ground. About 650 g of the powder was soaked in 2 L of cold distilled water. After 24 h, the solution was filtered and evaporated under vacuum and dried to a constant weight using a freeze-drier. The dry extract of the fruit was dissolved in distilled water instantaneously before treated the mice [20].

Preparation of cyproterone acetate:

Obtained cyproterone acetate anti-androgen from local pharmacies and called Androcur as a trading name and equipped from a company of a subsidiary of Filiale de Schering AG Germany, 20 mg concentration for each disc. Dissolved the drug used in this study in absolute ethyl alcohol and left exposed to the air until drought then added to the powder pure corn oil. solute each disc in 12.5 ml from corn oil to obtained 20 mg/ml this study in absolute ethyl alcohol and left exposed to the air until drought then added corn oil and calculated required concentrations to conduct experiments depending on the dose given to human[21].

Animals of the experiment:

In the current study, 40 male white mice have used range ages from used 2-3 months. The mice were provided with eating and water *adlibitum.*

Experimental protocols:

Mice were randomly divided into eight groups each contains 5 animals treated daily with one milliliter orally as follows:

1-First control group: treated with distilled water for 21 days, as a negative control.

2-Second control group: treated with corn oil for 21 days, as a positive control.

3-Third group: treated with cyproterone acetate 5 mg/kg/BW for 21 days.

4-Fourth group: treated with cyproterone acetate 20 mg/kg/BW for 21 days.

5-Fifth group: treated with cyproterone acetate 5 mg/kg/BW and crude date extract 60 mg/kg/BW for 21 days.

6-Sixth group: treated with cyproterone acetate 5 mg/kg/BW and crude date extract 120 mg/kg/BW for 21 days.

7-Seventh group: treated with cyproterone acetate 20 mg/kg/BW and crude date extract 60 mg/kg/BW for 21 days.

8-Eighth group: treated with cyproterone acetate 20 mg/kg/BW and crude date extract 120 mg/kg/BW for 21 days.

Animals were sacrificed 24 hours after of the last dose, use diethyl ether to drugged mice, open the abdominal cavity and remove the liver, then fixed the fresh small pieces of each mouse liver in the formalin solution 10 % until the histological preparation.

Histological study:

Ordinary histological processing is prepared for the liver in order to study the histopathological changes that may be found in the experimental groups as compared with negative and positive control groups. According to[22], the processing steps and staining technique was as follow: small pieces of livers were dehydrated using a graded ethanol series, subsequently emb.edded in paraffin, wax blocks were cut by the microtome to prepare 5μm thick sections and stained with hematoxylin after deparaffinization of sections in xylene and hydrated in progressive descending ethanol series and stained with eosin after the washing and differentiate,

then wash again, dehydrate, cleared in xylene and mount with Canada balsam on glass slide for light microscopic examination.

Results and Discussion

Several studies have indicated in Liver enzyme aberration have in experimental animals treated with cyproterone acetate [23]. This our study showed treated mice of 5 mg/kg body weight of CPA (group 3, fig. 1) revealed degeneration and necrotic changes in hepatocytes, sections of liver of CPA 20 mg/kg/BW treated mice (group 4, fig. 2) showed congested blood vessels, necrosis in hepatocytes and moderate inflammatory cell infiltration in the portal triad as compared to other treated groups. The hepatotoxicity depend on both the dose and the duration of xenobiotics exposure will impact the type and grade of toxicity, there is often susceptibility to the toxicity based on the intralobular site of hepatocytes for xenobiotics that immediately affect the liver and hepatocellular necrosis, evidence of necrosis is generally apparent within forty-eight hour or previously[24].

One study noted an increase in serum aspartate aminotransferase (AST) and alanine aminotransferase. (ALT) activities in all patients with inprogress prostate cancer who are treated with CPA-induced liver damage, in 91% of those cases, the type of hepatic injury was hepatocellular damage. This damage is frequently involved hepatocytes damage that is associated with an elevated ALT level (25). It is generally synched progress that hepatocellular hypertrophy may be a serious qualitative metric, but classify the severity of hypertrophy is less accurate than relying on liver weight or quantitative measuration of enzyme induction. There is a substantial relationship between hepatotoxicity and enzyme induction with clinical pathology measurements are described (26). Changes may occur in liver histology without enzyme reduction but include fluid aggregation, fatty change in hepatocytes, inflammatory cell infiltration, fibrosis, and probably granuloma formation (27).

Examination of stained liver sections of animals treated with 5 mg/kg/BW of CPA and crude aqueous palm date fruit extract 60 mg/kg/BW (group 5, fig.3) showed a noticeable degeneration in hepatocytes, sections of the liver of CPA 5 mg/kg/BW and crude palm date fruit extract 120 mg/kg/BW treated mice (group 6, fig. 4) revealed congested blood vessels and necrosis of hepatocyte as compared with CPA treated mice with reducing severity in two groups. "The mechanism by which the aqueous date palm fruit extract induces its hepatoprotective activity versus oxidative damage caused by any drug is not clear. However, it is potential that polyphenolic compounds (flavonoids, anthocyanins, and phenolic acids), and trace elements (selenium, copper, zinc, and manganese), an extension to vitamin C present in the date palm fruit are the responsible compounds for this protection (28; 29; 30).

One of the studies conducted on the aqueous extract of date fruits It acts as an antioxidant and the antimutagenic activity, Where this extract on the inhibition of lipid peroxidation and protein oxidation and also by the aptitude to scavenge superoxide and hydroxyl radicals [31]. In addition to that, there are many studies indicate the hepatoprotective activity of any drug is the ability of its components to block the aromatase activity of cytochrome P-450. On that basis, it is proposed that flavonoids in Phoenix dactylifera could be a factor contributing to its hepatoprotective ability through inhibition of cytochrome P-450 aromatase [32].

Tissue sections of the liver of CPA 20 mg/kg/BW and palm date fruit extract 60 mg/kg/BW treated mice (group7, fig. 5) showed congested blood vessels and severe degeneration in hepatocyte, in the liver of animals treated with CPA 20 mg/kg/BW and palm date fruit extract 120 mg/kg/BW (group 8, fig. 6) Although comparative studies indicated that dried date palm fruit with phenolic content was higher than fresh date palm fruits[33] . However, the water extract of dried palm fruit used in this study did not have the ability to preserve the liver from the toxic effect of the drug CPA especially at a high dose. The direct effect of cyproterone is attributed to increased of hepatocytes of placental glutathione S-transferase, which are believed preneoplastic elements[34-35]. Where it worksGrowth Factor-beta 1 (TGF) expression on the induction of apoptosis might account for both the liver damage and the expansion of liver tumors observed after giving a drug of CPA [36].

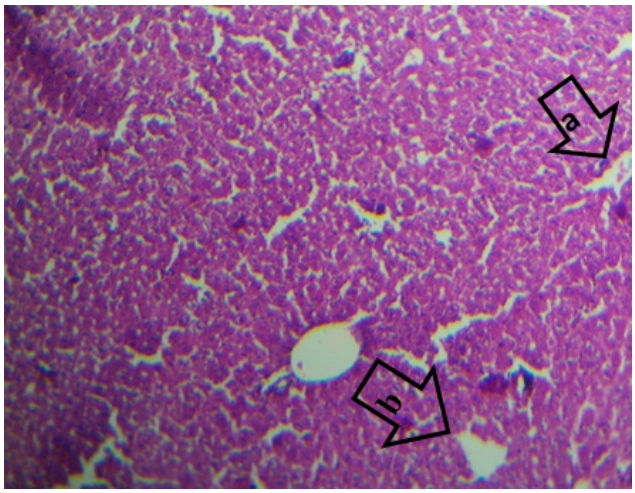


Figure-1: Histological liver section of (CPA) 5 mg/kg, group showed (a)Marked activation kuppfere cell . (b) degeneration of the hepatocyte necrosis of hepatocyte.

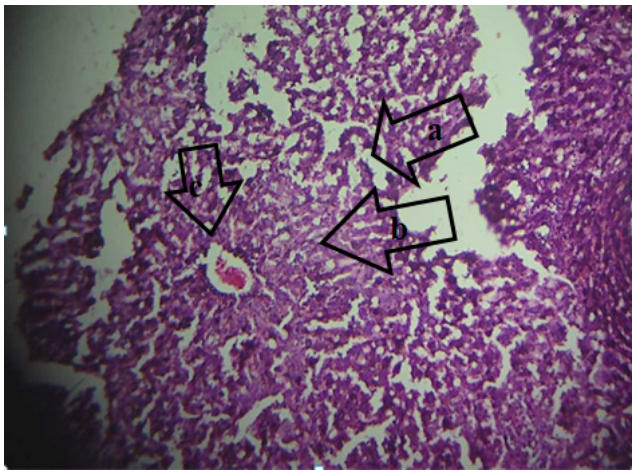


Figure-2: Histological liver section of (CPA) 20 mg/kg group showed (a) sever degeneration of hepatocyte (b).hypertrophied hepatocytes with deeply stained shrunken nuclei .(c) congested blood vessels.

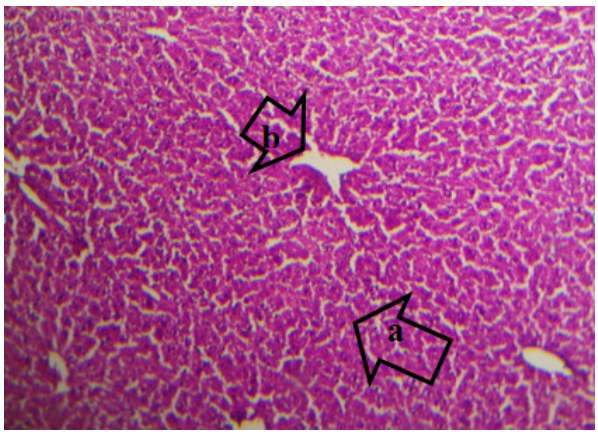


Figure-3: Histological liver section of (CPA) 5 mg/kg + 60 mg/kg date palm extract, group showed (a) Some hepatocytes were free from nuclei and others contained pyknotic nuclei.

Figure-4: Histological liver section of (CPA) 5 mg/kg + 120 mg/kg date palm extract, group showed (a) moderate hypertrophy of cells and (b) moderate hemorrhagic area .

Figure-5: Histological liver section of (CPA) 20 mg/kg + 60 mg/kg date palm extract, group showed sever degeneration of hepatocytes (b) and multihemorrhagic Areas.

Figure-6: Histological liver section of (CPA) 20 mg/kg+120 mg/kg date palm extract, group showed (a) congested blood vessel (b) necrosis of hepatocyte (c) moderate inflammatory cells infiltration in the portal triad.

Conclusion

the present study has shown that CPA has a toxic effect and some histopathological changes have been detected, so care should be taken when CPA is prescribed as antiandrogenic treatment.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Levesque H, et al. Fulminant hepatitis due to cyproterone acetate. The Lancet. 1989; 333(8631): 215- 216.
2. Kaplowitz N. Drug-induced liver injury: Clinical Infectious Diseases. (2004); (Supplement_2): S44-S48.
3. Murphy B. J., & Collins, B. J. . Severe hepatitis and liver failure induced by cyproterone acetate. Australian and New Zealand journal of medicine, 1996; 26.5: 724-724.
4. Kacar S, Akdogan M, Kosar Y, Parlak E, Sasmaz N, Oguz P, Aydog G. Estrogen and cyproterone acetate combination-induced autoimmune hepatitis. J. clin. Gastro. 2002; 35(1): 98-100.
5. Drakos P E, Gez E, Catane R. Hepatitis due to cyproterone acetate. Eur. J. Cancer. 1992; 28(11): 1931-1932.
6. Garty B Z, Dinari G, Gellvan A, Kauli R. Cirrhosis in a child with hypothalamic syndrome and central precocious puberty treated with cyproterone acetate. Eur. J. pediatr. 1999; 158(5): 367-370.
7. Rüdiger T, Beckmann J, Queiber W. Hepatocellular carcinoma after treatment with cyproterone acetate combined with ethinyloestradiol. The Lancet. 1995;(345)8947: 452-453.

8. Marwat SK, Khan MA, Rehman F, Bhatti IU. Aromatic plan species mentioned in the Holy Qur'an and Ahadith and their ethnomedicinal importance. Pak J Nut. 2009; 8: 1472-1479

9. Abdelouahhab Z, Arias-Jimenez E J. Date palm cultivation. Food and Agriculture Organization (FAO), 1999.

10. Khare C P. Indian Medicinal Plants An Illustrated Dictionary Springer Science. Berlin p g. Business Media. 2007: 699-700.

11. AL-FARSI, Mohamed A L, Chang Y. Nutritional and functional properties of dates: a review. Critical reviews in food science and nutrition. 2008;(48) 10: 877-887.

12. REGNAULT-ROGER, Catherine, et al. High performance liquid and thin-layer chromatographic determination of phenolic acids in palm (Phoenix dactilifera) products. Food Chem. 1987, 25(1): 61-71.

13. Mahran G H, Abdel–Wahab S M, Attia A M. A phytochemical study of date palm pollen. Planta Medica, 1976; 29(02): 171-175.

14. Al-Shahib W, Marshall RJ. The fruit of the date palm: Its possible use as the best food for the future? Int J Food Sci Nutr. 2003;54(4):247–59.

15. Garcia B, Marco J A, Seoane E, Tortajada A. Triterpenes, waxes, and tricin in Phoenix canariensis. J.Nat.Prod. 1981;44(1): 111-113

16. Saafi EB, El Arem A, Issaoui M, Hammami M, Achour L. Phenolic content and antioxidant activity of four date palm (Phoenix dactylifera L.) fruit varieties grown in Tunisia. Int J Food Sci Technol. 2009;44(11):2314–9.

17. Hasan M Z, Kane CL , Colloquium: topological insulators. Rev. Mod. Phys. 2010; 82: 3045-3067.

18. Al-Qarawi A A, Mousa H M, Ali B H, Abdel-Rahman H, El- Mougy, S. A. Protective effect of extracts from dates (Phoenix dactylifera L.) on carbon tetrachloride-induced hepatotoxicity in rats. Int J Appl Res Vet Med.2004; 2(3): 176-180.

19. Bahmanpour S, Talaei T, Vojdani Z , Panjehshahin M R, Poostpasand A, Zareei S, Ghaeminia M. Effect of Phoenix dactylifera pollen on sperm parameters and reproductive system of adult male rats. IJMS.2015; 31(4)).

20. Jahromi AR, Rasooli R , Kamali Y, Ahmadi N, Sattari , E. Short-term effects of date palm extract (Phoenix dactylifera) on ischemia/reperfusion injury induced by testicular torsion/detorsion in rats. Pharmacogn. Res. 92017;(1): 69.

21. Abd-Al-Ameer E H. The Effects of Cyproterone Acetate on the Sexual Performance and Fertility of the White Rats Rattus rattus. 2008 .Ph D. thesis. College of sciences. The University of Babylon.

22. Bancroft J D, Gamble M. Theory and practice of histology techniques. In: :. Churchill Livingstone Elsevier, 2008: 83-134.

23. Blake J C, Sawyerr A M , Dooley, J S, Scheuer PJ, McIntyre N. (1990). Severe hepatitis caused by cyproterone acetate. Gut.1990 ; 31(5): 556-557

24. EvansJ. Principles of toxicological pathology: Edited by John R. Glaister T. Food and Chem Toxicol. 1987; 25(4):337-338.

25. Kasper P. Cyproterone acetate: a genotoxic carcinogen. Pharmacol Toxicol. 2001;88:223–231.

26. Antoni M, Bourlière M, Toullec J, Maillot A, Botta-Fridlund D, Gauthier A. Fatal subfulminant hepatitis caused by cyproterone acetate Gastroenterol Clin Biol. 1991;15:772–773.

27. Evans J G., Lake B G. The digestive system. II: The hepatobiliary system. Target organ pathology: a basic text, 1998: 61-97.

28. Hong Y J, Tomas-Barberan F A, Kader A A , Mitchell A E. The flavonoid glycosides and procyanidin composition of Deglet Noor dates (Phoenix dactylifera). J. Agric. and food chem.2006; 54 (6): 2405-2411.

29. Allaith AAA. Antioxidant activity of Bahraini date palm (Phoenix dactylifera L.) fruit of various cultivars. Int J Food Sci Technol. 2008;43(6):1033–40.

30 Saafi EB, El Arem A, Issaoui M, Hammami M, Achour L. Phenolic content and antioxidant activity of four date palm (Phoenix dactylifera L.) fruit varieties grown in Tunisia. Int J Food Sci Technol. 2009;44(11):2314–9.

31. Vayalil, P. K. (2002). Antioxidant and antimutagenic properties of aqueous extract of date fruit (Phoenix dactylifera L. Arecaceae). Journal of Agricultural and Food Chemistry, 50(3), 610-617.

32. Kowalska M T, Brandt M E, Puett D. Inhibition of cytochrome P-450 aromatase activity by plant extracts. Planta Medica,1990; 56(06):675-675.

33. Al-Farsi, M.; Morris, A. and Baron, M.(2007).

Functional Properties of Omani dates (Phoenix dactylifera L.). Acta Hort., 736, 479-487.

34. Fasciati R, Maier P. EGF- and CPA-induced mitogenic stimuli are differentially down-regulated by TGF-β1 in cultured rat hepatocytes. Carcinogenesis. 1997;18(5):911–7.
35. Low-Baselli A. Initiated rat hepatocytes in primary culture: a novel tool to study alterations in growth control during the first stage of carcinogenesis. Carcinogenesis. 2000;21(1):79–86.
36. Oberhammer FA, Qin HM. Effect of three tumour promoters on the stability of hepatocyte cultures and apoptosis after transforming growth factor-β1. Carcinogenesis. 1995;16(6):1363–71

Molecular Detection of *Pseudomonas aeruginosa* and its Relationship with Multidrug Resistance and Transposons

Wathiq Abbas Hatite Al-Daraghi[1], Mohammed Sattar Abdulkadim Al-Badrwi[1], Haider Rida Jassim[1]

[1]*Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq.*

Abstract

P. aeruginosa is currently one of the most frequently nosocomial pathogens and the infections due to this organism are often difficult to treat due to antibiotic resistance. *P. aeruginosa* is an important pathogen in hospitalized patient's causative to their morbidity and mortality due to its multiple resistance mechanisms. Therefore, as a therapeutic option becomes restricted, the search for a new agent is a priority. One hundred and fifty samples were collected from different sources, divided into two main groups: clinical (80)samples and (70) hospital environment samples as a Nosocomial, collected all from October to the December of the year 2018. All of these samples were cultured by specific and differential media, Forty (40) isolates of *P.aeruginosa* bacteria were identified by using microscopic examination, biochemical tests. The identification of 40 isolates of *P.aeruginosa* bacteria confirmed by VITEK-2 system. A molecular detection the presence of *Tnp-R* gene in *P. aeruginosa* bacteria by conventional PCR to detect the Transposons and their relationship with multiple resistance of bacteria.

Keywords: *Multidrug Resistance; P. aeruginosa; and PCR*

Introduction

Pseudomonas aeruginosa is a Gram's negative opportunistic pathogen has emerged as one of the most problematic of the nosocomial pathogens; considered multi-resistant infections in both community and hospital settings, It causes infections in cancer, burn, urinary tract, surgical wound, eye, blood, ear infection, sepsis cystic fibrosis, and (ICU) (1). Because of it's an extremely ubiquitous organism and abundantly found in soil, water, plants, humans, animals, and in a hospital setting. P. aeruginosa is a common pathogen in hospital particularly in ICU although it has the ability to colonize healthy subjects, in addition to, bacterial exposure to some antibiotic classes may potentially induce endogenous resistance-conferring mutation in bacterial genes that encode drug targets (2). It has been progressively clear that resistance expansion in P. aeruginosa is their contexts with mutations in genes encoding porins, efflux pump, penicillin-binding proteins, and chromosomal β-lactamases, all contributing to resistance to β-lactamases, carbapenems, aminoglycoside, and quinolones(3). P. aeruginosa is an important pathogen in hospitalized patient's causative to their morbidity and mortality due to its multiple resistance mechanisms. Therefore, as a therapeutic option becomes restricted, the search for a new agent is a priority (4). The pathogenicity of P. aeruginosa is largely caused by multiple bacterial virulence factors and genetic flexibility enabling it to survive in a varied environment. A number of these factors aid colonization, while others allow bacterial invasion(5). Antibiotic resistance in bacteria has reached a near-crisis point in nosocomial health care, with many bacterial isolates now multi-resistant as a result of the presence of additional DNA element. Earlier studies have shown that genes for resistance markers do occur on plasmids and they can be transferable, and most of them have demonstrated it by plasmid curing experiments alone. Resistance gene can occur on chromosomes, transferable plasmid, Transposons or jumping gene and specialized transposons called integrons that can assemble multiple resistance genes into the cassette (6, 7).

Materials and Method

- **Specimens' Collection:**

During the period extended from October to the December of the year 2018, One hundred and fifty samples, divided into two main groups: (80)clinical samples and (70) samples hospital environment as a Nosocomial, were collected from hospitals. Clinical samples included: Urine samples from Urinary Tract Infections (UTI)patients, exudate samples from wounds

of the burn units patients, stool samples, Sputum samples from Cystic Fibrosis(CF) patients and Ear Swabs. The Nosocomial samples included many Nosocomial sources: Intensive Care Unit, Operations Hall, Birth Hall, Burning Hall, Devices and medical equipment, and hospital bed rooms.

- **Bacterial Isolation:**

In this study, the identification of 150 samples, we got 40 isolates of *P. aeruginosa* was performed by incubating these clinical and nosocomial isolates on different agar media (Nutrient agar ,Blood agar ,Maconcky agar, and Cetrimide agar which are a selective media for *Pseudomonas spp.*) and the incubation at 37˚C for 24 hrs. Forbes *et al*. [8].

- **Bacterial Identification:**

Identification of *P. aeruginosa* was confirmed by microscopically examination showed that it was single cells, a rod shape, not- spore-forming, and gram-negative, these results mention that this isolates may belong to *P. aeruginosa* growth on Cetrimide agar for characterization of *P. aeruginosa* such as mucoid, smooth in shape with flat edges and elevated center, creamy green colour and have a fruity odour.

- **PCR amplification:**

DNA template of all isolates was prepared by boiling method (30 min in 100°C). The DNA of isolates was targeted for the *bla*OXA-1 gene using the primers (Z.Tavajjohi, *et al* ., Iran)[9] listed in Table 1 and for the *Tnp-R* gene using the primers (Altaliby S. and Aldraghi w. ., Iraq) listed in Table 1. A reaction mixture (25 µl) contained 2 µl of DNA, 1 µl of each primer, 12.5 µl of Master Mix 2X(Z.Tavajjohi, *et al* ., Iran) and (Altaliby S. and Aldraghi w. ., Iraq), and 8.5 µl of Nuclease Free Water. The experiment was continued according to the following program: initial denaturation at 94°C for 5 minutes, followed by 30 cycles at 94°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute and a final extension at 72°C for 5 minutes. The PCR products were analyzed using gel electrophoresis (1% agarose) and stained with safe dye and visualized by Gel Doc apparatus (BioRad, USA) (Table 2).

Table (1): Primers used in this study.

Gene	Primer Sequence		Product size (bp)	References
bla OXA-1	F	5`-AGCCGTTAAAATTAAGCCC-3`	908	Z.Tavajjohi, et al ., 2011
	R	5`-CTTGATTGAAGGGTTGGGCG-3`		
Tnp-R	F	5`-TTTTGGTTATGCGCGGGTC-3`	545	Altaliby S. and Aldraghi w. .,2018
	R	5`-AGGCCCTTTCGTCTTCAAGA-3		

Table (2): Condition of PCR Reaction for *bla*OXA-1 and *Tnp-R* genes of *P.aeruginosa* .

Steps	Temperature	Time	Number of Cycle
Initial Denaturation	95°C	5 min.	1
Denaturation	95°C	30 Sec.	30
Annealing	55°C	30Sec.	
Extension	72 °C	30Sec.	
Final extension	72 °C	7 min.	1
Hold	4 °C		1

Results and Discussion

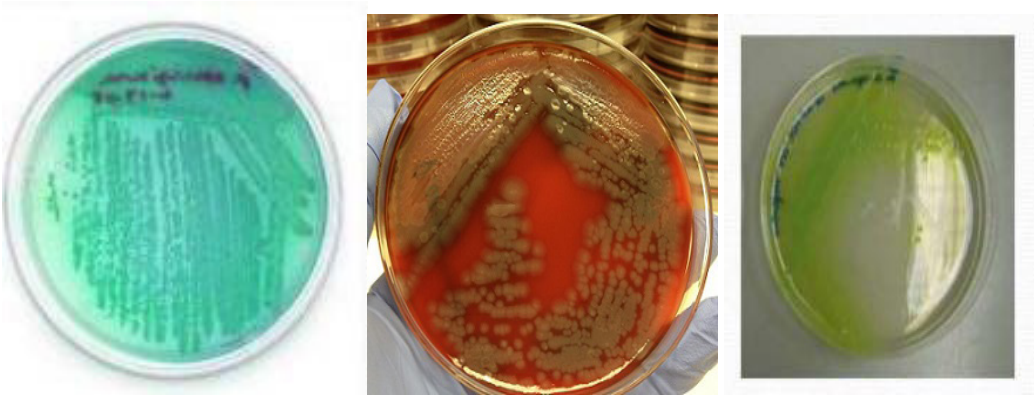
Isolation and Identification of *P. aeruginosa*:

One hundred and fifty samples clinical and Nosocomial samples were analyzed for the presence of *P. aeruginosa*, and the results of bacterial isolation and identification revealed the detection of forty (40) isolates of *P. aeruginosa*.

Biochemical Tests:

Some biochemical tests were performed for more validation. showed 40 isolates of *P.aeruginosa* provided a by some biochemical tests,

results showed positive results for oxidase test, catalase test, motility test, , and production of *B*-hemolysis while (40) isolations negative results to citrate utilization tests, indole production and urease production tests negative to Gram's stain and capable of growing on cetrimide agar as yellow greenish colonies (at 42˚C for 24 hrs.). (Figure 1).



A B C

Figure (1): *P. aeruginosa* colonies on (A) Cetrimide agar, (B) and Blood agar, and (C) Nutrient agar after 24 hours of incubation at 37ºC.

At the species level, *P. aeruginosa* has a wide growth temperature range, optimum growth at 37°C. Slower growth rates are seen at 4˚C. *P. aeruginosa* is distinguishable from other clinically *Pseudomonas* spp. by its capability for growth at 42˚C. They also grow well at pH range 6.6-7.0. It was tolerant of a wide variety of physical conditions, including temperature and pH[10]. Also, it was resistant to high concentrations of salts and dyes. It is typically given a positive result to the oxidase test and catalase. It does not ferment carbohydrates, but many strains oxidize glucose [11].

The identification was performed with the automated VITEK -2 system using the GN-ID cards which contains 64 biochemical tests, from (40) isolate of *P. aeruginosa* ,(40) positive result of the *P. aeruginosa* demonstrated.

Distribution of *P. aeruginosa* according to Type of Specimens

According to table (3), Out of (80) clinical samples of burns, sputum, urine, stool, ear and wound, 28(70%) isolates were positive to clinical *P. aeruginosa* and the percentage of the positive results from (70)Nosocomial samples were 12 (30%), as reported in (Table 4).

Table (3): Distribution of *Pseudomonas aeruginosa* isolates in clinical samples.

Site of samples	Numbers of sample and Percentage
burn swab	2 (5 %)
wound swab	5 (12.5%)
ear swab	11 (27.5%)
Sputum	4 (10%)
Urine	6 (15%)
Total	28 (70%)

Table (4): Distribution of *Pseudomonas aeruginosa* isolates in Nosocomial samples

Site of samples	Numbers of sample and Percentage
ICU	2 (5%)
Operations Hall	4 (10%)
Birth Hall	2 (5%)
Burn Hall	2 (5%)
Devices and medical equipment	2 (5%)
Total	12 (30%)

The low percentage was found in burn specimens which accomplished 5%. Results obtained reported that the highest percentage of *P.aeruginosa* from ear swab (27.5%) was in opposite with our results. In comparison with Nosocomial isolates of *Pseudomonas aeruginosa* the highest percentage isolation of Operations Hall (4%) where are other isolate reported low present in comparison with clinical findings.

P. aeruginosa is pathogenic only when introduced into areas devoid of normal defences, the bacterium attaches to, and colonizes the mucous membranes or skin, invades locally and produces systemic disease. *Ps. aeruginosa* infects healthy tissues rarely, but, when defences are compromised, it can infect virtually all tissues. This explains why most infections are nosocomial[12]. These infections are Pneumonia, Osteomyelitis (related to Wounds, Immunocompromised patients, Burn-wound infections, Urinary tract infections, Endocarditis, external otitis and Tissue layer infections [13].

Genomic DNA Extraction:

Using a Genomic DNA Purification Kit (Promega), Genomic DNA was extracted from (40) *P. aeruginosa* isolates that were confirmed as bands by gel electrophoresis. Quantus Fluorometer was used to detect the concentration of extracted DNA in order to detect the goodness of samples for downstream applications. all the isolates had DNA concentration between (10-40 ng/µl) by Quantus Fluorometer.

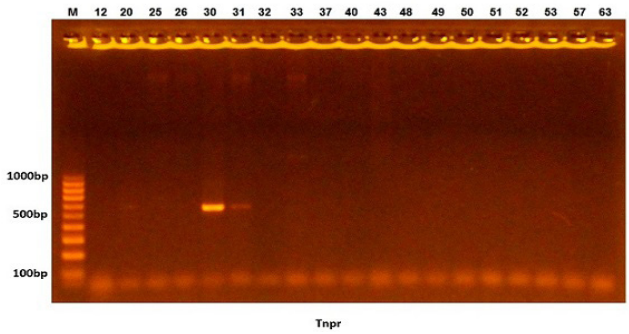
Molecular Detection of *P. aeruginosa* and co-strains by $bla_{oxa1\ like}$ gene:

The result of PCR analysis concerning of the found the blaoxa-1 in (10) positive isolates, showed that studies P. aeruginosa possess the blaOXA-1 like gene from 10(25 %) isolates positive showed 4 (40%) from clinical isolates and 6 (60 %) from nosocomial.

The ESBL*bla*oxa-1 of *P. aeruginosa* isolates exhibited co-resistance against most of the antibiotics tested. This is consistent with most of the recent findings [14]. The *bla*OXA-1 ESBLs provide *P. aeruginosa* with an additional powerful resistance mechanism with potentially serious clinical implications, including limitation of the therapeutic options. ESBLs manufacturing organisms create distinctive challenges to clinical microbiologists, clinicians, infection control professionals and scientists engaged in finding new antibacterial agents [15].The development and spread of ESBLs are most likely caused by the overuse of expanded- spectrum Ciprofloxacin in the hospital setting. Proper infection management practices and barriers are essential to stop spreading and outbreaks of ESBL-producing microorganism [16].

Molecular Detection of Transposons of *P. aeruginosa*:

The result of PCR analysis concerning of the found the *Tnp-R* gene in (2) (12%) positive isolates from 10 *bla*oxa1 positive *P. aeruginosa* isolates ,which identified the presence of Transposon in this bacteria as reported in figure(2).



Figure(2): Agarose gel electrophoresis (1% agarose, 100Vol / mAmp for 75 min.) of PCR amplification products (Tnp-R gene) at 545bp for P. aeruginosa. lanse 30and 31 show positive results for P. aeruginosa; ladder 100 bp DNA marker.

Two strains of nosocomial isolates of *P. aeruginosa* exactly show bands for *Tnp-R* gene which represent the nosocomial sample number (30) and (31) were collected from the hospital's operation hall and birth rooms respectively.

Strains of bacteria resistant to antibiotics, particularly those that are multi-drug resistant, are an increasingly major health care problem around the world. [17]. This is achieved through the cooperative activities of mobile genetic elements able to move within or between DNA molecules, which include insertion sequences, transposons, and gene cassettes/integrons, and those that are able to transfer between bacterial cells, such as plasmids and conjugative elements. Together these types of mobile genetic elements play a central role in facilitating horizontal genetic exchange and therefore promote the acquisition and spread of antibiotic resistance genes in both Gram-negative and Gram-positive bacteria, focusing on the group of organisms (*S. aureus , K. pneumonia , A. baumannii , P. aeruginosa , Enterobacter spp., and Escherichia coli*), which have become the most problematic hospital pathogens [18].

Whereas one or two classes are left in presence of Integron and/or Transposons, extensive drug resistance **(XDR)**. **MDR/XDR** has appeared in *P. aeruginosa*, *Acinetobacter baumannii*, *E. coli*, and *K. pneumonia,* producing extended-spectrum β-lactamases (ESBL), vancomycin-resistant *enterococci, Enterococcus faecium* (VRE), MRSA, vancomycin-resistant *Staphylococcus aureus* VRSA, *Salmonella enterica* serovar *Typhimurium*, *Shigella dysenteriae*, and *Burkholderia* [19-21].

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Tille, P. (2015). Bailey and Scott's diagnostic microbiology-E-Book. *Elsevier Health Sciences.*
2. Odumosu, B.T., Adeniyi, B.A. and Chandra, R Analysis of integrons and associated gene cassettes in clinical isolates of multidrug resistant *Pseudomonas aeruginosa* from Southwest Nigeria. *Ann Clin Microbiol Antimicrob*; .(2013). 12(29) doi: 10.1186/1476-0711-12-29.
3. Barbier, F**.,** Andremont, A., Wolff, M., Bouadma, L., Hospital-acquired pneumonia and ventilator-associated pneumonia: recent advances in epidemiology and management. *Curr Opin Pulm Med* (2013). 19, 216–228.
4. Tacconelli, E., Magrini, N., Carmeli, Y., Harbarth, S., Kahlmeter, G., Kluytmans, J., Mendelson, M., Pulcini, C., Singh, N., Theuretzbacher, U., (2017). The global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. *World Health Organization.*
5. Todar, K. (2008). *Pseudomonas aeruginosa*. Todar's Textbook of Bacteriology, 1.
6. Khajuria, A., Praharaj, A.K., Kumar, M. and Grover, N, The emergence of NDM – 1 in the clinical isolates of *Pseudomonas aeruginosa* in India. *J Clin Diagn Res* .(2013) 7, 1328–1331.
7. Cavalcanti, F.L., Mirones, C.R., Paucar, E.R., Montes, L.A., Leal-Balbino, T.C. Mutational and acquired carbapenem resistance mechanisms in multidrug resistant *Pseudomonas aeruginosa* clinical isolates from Recife, *Brazil. Mem Inst Oswaldo Cruz* (2015) 110, 1003–1009.
8. Forbes, A.; Sahm, F. and Weissfeld, S. (2007) Bailey and Scott 's, Diagnostic Microbiology. International Twelfth Edition , page 172,174, 176, 254, 265, 323, 403, 834.
9. Zahra Tavajjohi1, Rezvan Moniri and Ahmad Khorshidi, Detection and characterization of multidrug resistance and extended-spectrum-beta-lactamase-producing (ESBLS)*Pseudomonas aeruginosa* isolates in teaching hospital, Department of Biology, Faculty of Science, Alzahra University, Tehran, Iran, African *Journal of Microbiology Research* Vol. (2011). 5(20), 3223-3228.
10. Moore, N.M. and Flaws, M.L. Antimicrobial resistance mechanisms in *Pseudomonas aeruginosa .Clin Lab Sci*; (2011). 24: 47-51.
11. Kipnis, E., Sawa, T., and Wiener-Kronish, J. Targeting mechanisms of *Pseudomonas aeruginosa* pathogenesis. *Medicine et maladies infectieuses*, (2006) 36(2), 78-91.
12. Jones RN. Microbial Etiologies of Hospital-acquired bacterial Pneumonia and Ventilator-associated Bacterial Pneumonia. *Clin Infect Dis*; (2010). 51(l): 81-87.
13. Sibhghatulla Shaikh, Jamale Fatima, Shazi Shakil, Syed Mohd. Danish Rizvi, and Mohammad Amjad Kamal. Prevalence of multidrug-resistant and extended-spectrum beta-lactamase producing

Pseudomonas aeruginosa in a tertiary care hospital, *Saudi J Biol Sci*.; (2015). 22(1): 62–64.

14. Hirsch EB, Impact of multidrug-resistant *Pseudomonas aeruginosa* infection on patient outcomes, Expert Rev Pharmacoecon Outcomes Res. 2010 Aug; 10(4): 441–451.
15. Bandekar, N., & Vinodkumar, C. S. Beta-lactamases mediated resistance amongst gram-negative bacilli in Burn Infection Int J Biol Med Res. 2011; 2(3): 766-770
16. Joumana. N., Samaha-Kfoury, and Araj, G. F. Recent developments in β lactamases and extended spectrum β lactamases. *Biology molecular j,* (2003). 327(7425), 1209-1213.
17. Partridge SR1and Kwong S.M. (2018). Mobile Genetic Elements Associated with Antimicrobial Resistance. Firth SE, *Clin Microbiol Rev*. **DOI:** 10.1128/CMR.00088-17
18. Walsh, C. (2000). Molecular mechanisms that confer antibacterial drug resistance. *Nature*, *406*(6797), 775.
19. Giedraitienė, A., Vitkauskienė, A., Naginienė, R. and Pavilonis, A. Antibiotic resistance mechanisms of clinically important bacteria. *Medicina*; (2011).47, 137–146.
20. Džidić, S., Šušković, J. and Kos, BAntibiotic resistance mechanisms in bacteria: biochemical and genetic aspects. *Food Technology and Biotechnology* .(2008). 46, 11–21.
21. Tal, L., and enzavi ,R ,A (2011).Developmental regulation of transposable elements in Wheat and Maize kernels (Doctoral dissertation, Weizmann Institute of Science).

Antifungal Activity of Silver Nanoparticles Using *Penicillium Chrysogenum* Extract Against The Formation of Biofilm for *Candida Glabrata*

Nada S. Salah[1], Thamer A.A. Muhsen[1], Mohssen H. Risan[2]

[1]College of Education for Pure Science / Ibn Al-Haitham, University of Baghdad/Iraq,
[2]College of Biotechnology / University of Al-Nahrain/Iraq

Abstract

The results showed that 71 isolates of *Candida* spp were isolated from patients with leukemia both women and men, Isolate 59 from *C. glabrata* while the number of isolates of *C.albicans*, *C.tropicalis*, *C.krusei* and *C.kefyer* were 6,3,2,1 respectively. The size of the nanoparticle was measured using AFM, The highest peak was 455 nm due to the presence of surface plasmons and another 243 nm wavelength, SEM showed the presence of particle of different sizes and distributed regularly and small silver nanoparticle. Effect of synergistic silver nanoparticle and antifungal agent (fuconazole) on the biofilm of *Candida glabrata*, capable of *C.gabrata* on adhesion of epithelial cells in the absence of silver nanoparticle and fluconazole, no adhesion between epithelial and yeast cells when adding silver nanoparticles, Decrease in surface adhesion between biofilm of the yeast and the epithelial cell when adding fuconazole, When collecting silver nanoparticles with fluconazole and adding it to epithelial cells exposed to *C.glabrata*, It led to the inability of the yeast to adhere to epithelial cells and then died . All experiments showed the least significant differences at 0.001 level.

Key Words: *Penicillium chrysogenum, silver nanoparticle, Candida glabrata, antifungal.*

Introduction

Candida yeast is transformed from a saprophytic organism into a pathogen due to Candida's factors such as adhesion, protease production, phospholipids, hemolysin proteins, biofilm and germ tube formation, Pathogenesis also depend on the host's immune system[(1)]. One of the factors causing an increase in candidiasis is the chronic illness of people such as diabetes, weak immune system, malignant tumors, pregnancy and excessive use of antibiotics, which are factors for the emergence of infection[(2)]. *C.glabrata* is a mono-chromosome group (haploid) that has no dimorphic form and severe opportunism in the genitourinary system and in the bloodstream Candidemia is particularly prevalent in older people and infected with HIV[(3)]. *C.glabrata* is common in 15-20% of infections and many of its isolates are resistant to fungal antibiotics such as Amphotericin B and Fluconazol[(4)]. [(5)] indicated that yeast has the ability to form a biofilm which is environmentally important and helps them to survive as human pathogens by allowing them to escape host immunity mechanisms, resist antifungal and compete with other microorganisms, The formation of the biofilm is a key factor in species survival.

Penicillium chrysogenum is common in temperate and subtropical regions and is found in food products such as citrus and grains[(6)]. [(7)] noted that *P.chrysogenum* was widely used in the industry and in the treatment of certain plant wastes and the production of enzymes such as Polyamine Oxidae and Phospho-gluconate dehydrogenase. It also has a high potential for production of Penicillin antibody and the first commercially produced penicillin.

[(8)] noted that fungi contains some distinctive advantages when used as biosynthesis for the production of nanoparticle compared to bacteria by producing larger

Corresponding author:
Thamer A.A. Muhsen
E-mail: thamer.a.m@ihcoedu.uobaghdad.edu.iq

amounts of Mechanism of action of silver nanoparticles (AgNPs) against yeast by targeting the biofilms of *Candida glabrata*, Analysis of the active electron microscopy revealed that the interaction between nano-Ag and *C. glabrata* cells during AgNPs exposure leads to changes in membranes which can be observed as holes on the surface of the membrane and lead to cell death[9]. The technique of silver nanoparticles led to the movement of the drug into the tissues of the body, which was previously unreachable and was based on several factors including pH, temperature, solubility in the medicine, absorption of the surface-related drug and the spread of the drug through the matrix of nanoparticles[10].

Materials and Method

Collections of Samples: Collected130 clinical samples taken from patients of leukemia from the City of Medicine/ Leukemia Department in Baghdad City

Isolation of yeast: Placing 100 microliters of blood on the sabroud dextrose agar (SDA)[11].

Identification of *Candida*: For the purpose of diagnosing *Candida* was studied, Characterstions of Morphological[12], Purification of Colonies[13].

Virulence of factor test: The following experiments were performed, germ tube test (14). Biofilm formation test[15], Candida Chromgenic agar[16], the Vitek2 Compact System.

Identification of *Penicillium chrysogenum*: The fungus of *Penicillium chrysogenum* according[17].

Biomass of *Penicillium chrysogenum*: For the fungal biomass by[18].

Preparation of silver nanoparticles in *Penicillium chrysogenum*: The silver nanoparticles were composed by observing in kind the color change of the yeast from the transparent color to the dark brown color[19].

Characterization of nanoparticle using different microscopes:

A.Atomic Force Microscope: Use this microscope to find out the size of nanoparticles and monitor the bio-processing of nanoparticles and know the particle size[20].

B. UV-ViS Spectrophoto meter: The UV spectrometer is used to monitor the biotransformation of silver ions by means of UV spectroscopy of the reaction[21].

C. Scanning Electron Microscope: Use this microscope to determine the size and shape of the nanoparticles and to know the structural[20].

Studying the synergistic effect of nanoparticle and antifungal for the biofilm agent of the *Candida glabrata*: This technique was used to test epithelial cells of the mouth on adhesion the biofilm to *Candida glabrata* [22] as follows:

A- First treatment: Take 0.5 ml of sediment containing epithelial cells (control treatment).

B-Second treatment: Take 0.5 mL of the sediment containing the epithelial cells and add 0.5 ml of *Candida glabrata.*

C- Third treatment: Take 0.5 ml of sediment containing epithelial cells and add 0.5 ml *Candida glabrata* and 50 microliters of silver nanoparticles composed with *Penicisllium chrysogenum.*

D- Treatment 4: Take 0.5 ml of sediment containing epithelial cells and add 0.5 ml *Candida glabrata* and 100 mg of antifungal Fuconazole.

E- Treatment 5: Take 0.5 ml of sediment containing epithelial cells and add 0.5 ml *Candida glabrata* and add 100 mg of Fuconazole and add 50 microliters of silver nanoparticles with *Penicillium chrysogenum.*

Statistical analysis: Statistical Analysis System SAS (2012)[23].

Results and Discussion

Distribution of infected patients with candidiasis: The results showed that 71 isolates of *Candida* spp were isolated from patients with leukemia, both women and men, with 34 clinical cases of women. The 50-65 age group recorded 17 cases of 50% and 9 cases of the 20-30 years and 26.5%. The age group between 40-50 years recorded 5 cases and 14.7%. Finally, the age group of 17 years had the lowest rates of 8.8% and three clinical cases as in Table (1).

Table (1): Shows the distribution of women by age group Candidiasis patients due to leukemia.

Age group	No. of infected of women\34	%Percentage
50-65 years	17	50
40-50 years	9	26.5
20-30 years	5	14.7
Less than 17 years	3	8.8
Total	34	100%
Chi-Square (χ^2) P-value	---	13.594 ** 0.0036

** (P<0.01).

In the case of men, there were 37 clinical and positive cases of yeast *Candida* spp. The results showed positive results for yeast for leukemia patients in the age group 50-65 years in 14 cases and 37.9%, followed by age group 40-50 years and 11 cases and 29.7% The age group between 20-30 years, which was 7 cases and 18.9%. Finally, the lowest age group of 17 years recorded the lowest rates of 13.5% and 5 clinical cases table (2).

Table (2): shows the distribution of men by age group. Candidiasis patients due to leukemia

Age group	No. of infected of Men \37	%Percentage
50-65 years	14	37.9%
40-50 years	11	29.7
20-30 years	7	18.9
Less than 17 years	5	13.5
Total	37	100%
Chi-Square (χ^2) P-value	---	5.270 * 0.0530

* (P<0.05).

This is consistent with[24] which showed that Candidiasis is the fourth most common type of infection of the bloodstream and causes candidasis for patients in hospital. The increase in infection in these age groups is due to the availability of appropriate conditions such as immunodeficiency, long-term use of antibiotics and malignant tumors.

Identification of *Candida* spp.: Table (4), 71 isolates were obtained from clinical samples of women and men with leukemia, 59 *C. glabrata* from 71 isolates and 83.1%, while the number of isolates of *C.albicans*, *C.tropicalis*, *C.krusei* and *C.kefyer* were 6,3,2,1 respectively. These results were consistent with[25], indicating that *C.glabrata* was the second most common cause and 24% of Candida in the United States of America. In 2004 *Candida glabrata* was the main cause of Candidiemia, and mortality rates for *Candida glabrata* patients were detected. 50% in cancer patients, and 100% in bone marrow patients.

Table (4): shows the distribution of *Candida* isolated from patients with leukemia from women and men.

Candida spp	No. of Isolates of Leukemia/Women and Men	Percentage%
C.glabrata	59	83.1
C.albicans	6	8.5
C.tropicalis	3	4.2
C.krusei	2	2.8
C.kefyer	1	1.4
Total	71	100
Chi-Square (χ^2) P-value	---	177.662 ** 0.0001

** (P<0.01).**4.Biosynthesis of silver nanoparticles**

Combining the biomass of *Penicllium chrysogenum* with the silver nitrate solution results in color difference. This indicates the formation of silver nanoparticle by the presence of surface plasmon, which is consistent with[26]. The difference in color is due to the difference in the electron density of the nanoparticles that are different nano-size.

Characterization of silver nanoparticle.

Atomic Force Microscope (AFM) Results: The average square root value is equal to Root Mean Square = 2.12nm. The surface roughness of the membrane is

average Roughness = 1.54nm. This value is a proof of surface roughness, The particle size was found to be 18.83 nm. [27]showed that the nanoparticle were modified by *Fusarium graminaerum* in different sizes and measured using AFM and began with a diameter of 1 nm.

UV spectrophotometer results:

The highest peak was 455 nm and another 243 nm wavelength and the highest peak due to the presence of surface plasmons either the second peak may indicate the presence of tyrosine and tryptophan residues found in the protein released from the yeast. This is explained[28] suggests that the reduction of silver nitrate to silver nanoparticles can be easily by using the UV spectrometer because silver nanoparticles can absorb light in the visible area due to Plasmon surface.

Scanning Electron Microscope (SEM) Results:

Fig. (1) showed the presence of spherical particle of different sizes and distributed regularly and small silver nanoparticles, this supports[29]. That the surface of the plasmon reached a peak of 420 nanometers and that silver nanoparticles have a spherical shape.

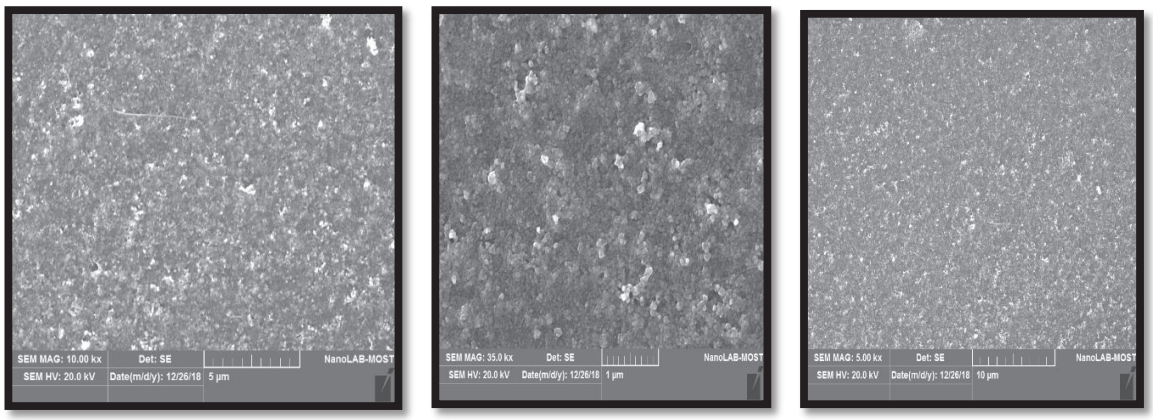


Figure (1) SEM for prepared silver nanoparticles.

Effect of synergistic silver nanoparticle of *Penicillium chrysogenum* and antifungal agent on the biofilm of *Candida glabrata*: Results showed the effect coefficients the synergy of silver nanoparticle particles For *Penicillium Chrysogenum* and antifungal agent against the *Candida glabrata* has different effects. Figure (5-A) shows the normal shape of the epithelial cells that represent the control treatment, between Figure (5-B) capable of *C.gabrata* on adhesion of epithelial cells in the absence of silver nitrate and antifungal. Figure (5-C) showed no adhesion between epithelial and yeast cells when adding silver nanoparticles with *Penicillium chrysogenum*. This indicates the inability of *C.glabrata* to adhere to the presence of silver nanoparticles. The exposure of epithelial cells with *C.glabrata* and antifungal fuconazole showed a decrease in surface adhesion between biofilm of the yeast and the epithelial cell as shown in Figure (5-D). When collecting silver nanoparticles with fluconazole and adding it to epithelial cells exposed to *C.glabrata*, Yeast inability was observed on adhesion with epithelial cell and then its death due to the presence of synergistic nanoparticle with fluconazole showed stable and strong antifungal activity as in Fig. (5-E). The results showed that silver nanoparticle have properties antifungal.It can also provide synergy with antifungal when evaluating the synergistic effect of silver nanoparticles and the fluconazole against the adhesion cells formed for the biofilm of *C.glabrata*, This is consistent with the study of silver nanoparticle antifungal such as floconzole against *Candida albicans* and a strong synergistic effect between silver nanoparticles and antifungal[30]. [31]showed that silver nanoparticles are associated with important cellular structures of proteins and DNA and cause cellular damage to yeast. [32]explain The association of the silver atoms with the thiol group (SH) in the enzymes, which change the composition and function of the enzymes in the cell membrane, which makes the adhesion ineffective.

Figure (2) A- Normal epithelial cells, B- Epithelial cells with *C.glabrata*, C- Epithelial cells with *C.glabrata* when adding silver nanoparticles, D- Epithelial cells with *C. glabrata* when adding Fluconazole, E- Epithelial cells with *C. glabrata* when adding silver nanoparticles with Fluconazole.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Yang YL. Virulence factors of *Candida* species. J Microbiol Immunol Infect. 2003; 36(4):223-228.
2. Brooks GF, Butol JS, Mrse SA. Jewets, Melmicka & Alberges medical microbiology. 24th. ed. Appleton. Lange, Asemon & Schusterco, Californinia. 2001.
3. Rodrigues CF, Rodrigues ME, Silva S, Henriques M. Candida glabrata Biofilms: How Far Have We Come? J Fungi. 2017; 3(1): 11.
4. Vazquez JA, Sobel JD. Candidiasis.Chapter 11 In: Clinical Mycology.Ed. WE Dismukes, PG, Pappas, JD Sobel. Exford University press. 2003; pp:519.
5. Silva S, Henriques M, Oliveira R, Williams D, Azeredo J. *In vitro* biofilm activity of non-Candida albicans Candida species. Curr Microbiol. 2010; 61:534–540.
6. Anderson B, Frisvad JC, Sondergard I, Rasmussen IS, Larsen LS. Association between fungal species and water damaged building. 2011.
7. Domsch K H, Gams W, Anderson TH. Compendium of soil fungi .Vol.I and II. Academic press. 1980 .PP : 941.
8. Mohanpuria P, Rana KN, Yadav SK. Biosynthesis of nanoparticles: technological concepts and future applications. J Nanopart Res. 2008; 10: 507–517.
9. Gajbhiye M, Kesharwani J, Ingle A, Gade A, Rai M. Fungus-mediated synthesis of silver nanoparticles and their activity against pathogenic fungi in combination with fluconazole. Nanomed Nanotechnol Biol Med. 2009; 5(4):382-386.
10. Son GH, Lee BJ. Mechanisms of drug release from advanced drug formulations such as polymeric-based drug-delivery systems and lipid nanoparticles. J Pharmaceut Invest. 2017.
11. Vandeppitte J, Engback K, Piot P, Hench CC. Basic laboratry procedures in clinical bacteriology. WHO. Geneva, Switzerland. 1991; V.85.
12. Murray PR, Baron EJ, Pfaller MA,Tenover FC, Yolken RH. Manual of clinical microbiology. 7th ed.Asm Press.Washngton. 1999.
13. Milan EP, Zaror L. Laboratory diagnosis of some types of fungi: Medical Mycology. Rio de Janeiro: Guanabara Koogan. 2004; 89-101.
14. Yan LJ, Thangthaeng N, Sumien N, Forster MJ. Serum dihydrolipoamide dehydrogenase is a labile enzyme. J Biochem Pharmacol Res. 2013; 1(1):30.
15. Naveen S, Deepak M, Divya D, Savita S. Evaluation of Congo Red Agar for Detection of Biofilm Production by Various Clinical Candida Isolates. J Evol Med Dental Sci. 2014; 59(3): 13234-13238.
16. Hospenthal DR, Beckius, ML, Floyd KL, Horvath LL, Murray CK. Presumptive identification of Candida species other than, *C. albicans*, *C. kusei* and *C. tropicalis* with the chromogenic medium CHORMagar Candida. Ann Clin Microbiol Antimicrob. 2006; 3(5): 1-10.
17. Ellis MB. Dematiaceous hyphomycetes. Kew, Surrey, U.K: Common Wealth Mycological Institute. 1971.
18. Kamiar Z, Seyedmohammad P, Arman S, Pouyan M, Keyvan P, Mohammad, JR, Ali AM. Biosynthesis and Characterization of Silver Nanoparticles by *Aspergillus* species. Biomed Res Int. 2016; 6(1): 6.
19. Abeer RM, Abd El- Aziz A, Monira RA, Saleh AE, Mohamed AM, Majrashic M. Green Synthesis of Silver Nanoparticles using *Aspergillus terreus*. Digest J Nanomat Biostruct. 2013; 8(3): 1215–1225.
20. Logeswari P, Silambrasan S, Abraham J. Synthesis of Silver nanoparticles using plant extract and analysis of their antimicrobial property.J. Saudi Chem. Soc. 2012.
21. Husseiny SM, Salah TA, Anter HA. Biosynthesis of size controlled silver nanoparticles by Fusarium oxysporum their antibacterial and antitumor activities. Beni-Suef Uni J Basic Appl Sci. 2015; 4(3):225-231.
22. Juliana PL, Fa´bio V, dos S, Pedro CG. Leonardo Marmo Moreira Mycopathologia. 2011; 171:93–101.
23. SAS. 2012. Statistical Analysis System, User's

Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc.

24. Zaoutis TE, Argon J, Chu J, Berlin JA, Walsh TJ, Feudtner C. The epidemiology and attributable outcomes of candidemia in adults and children hospitalized in the United States: a propensity analysis. Clin Infect Dis. 2005; 41: 1232-1239.
25. Li L, Redding S, Dongari-Bagtzoglou A. *Candida glabrata*, an emerging oral opportunistic pathogen. J Dent Res. 2007; 86: 204–215.
26. Netala VR, Bobbu PL, Ghosh SB, Tartte V. Endophytic fungal assisted Synthesis of Silver nanoparticles, Characterization, and antimicrobial activity. Asian J Pharm Clin Res. 2015; 8(3): 133-116.
27. Shafiq SA, Al-Shammari RH, Majeed HZ. Study of Biosynthesis silver nanoparticles by Fusarium graminaerum and test their antimicrobial activity. Int J Inno Appl Stud. 2016; 15(1): 43.
28. Rahimi G, Alizadeh F, Khodavandi A. Mycosynthesis of silver nanoparticles from Candida albicans and its antibacterial activity against Escherichia coli and Staphylococcus aureus. Trop J Pharm Res. 2016; 15:371–375.
29. Abdel-Rahim K, Mahmoudc S, Alic AM, Almaarya KS, Mustafaa MA, Husseinye SM. Extracellular biosynthesis of silver nanoparticles using *Rhizopus stolonifer*. Saudi J. Biol. Sci. 2017; 24(1): 208–216.
30. Singh M, Kumar M, Kalaivani R, Manikandan S, Kumaraguru AK. Metallic silver nanoparticle: a therapeutic agent in combination with antifungal drug against human fungal pathogen. Bioprocess Biosyst Eng. 2013; 36: 407-15.
31. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv. 2009; 27(1): 76–83.
32. Rai M., Ingle A. Role of nanotechnology in agriculture with special reference to management of insect pests. Appl. Microbiol. Biotechnol. 2012; 94(2): 287–293.

Study of Epidemiological Factors According to the Positive Response of Igg of Patients Infected with *Blastocytsis hominis* in Diyala province, Iraq

Muna Hameed Ahmed Alazzawi[1], Nabaa Ali Jasim[1], Masar H.Ismail[3]

[1]Lecturer: Directorate of General for the Education of Diyala, Iraq, [2]Lecturer: Department of Community Health Technical Institute, Baquba, Iraq, [3]Lecturer: Department of Biology. College of Education for Pure Sciences, Diyala University, Diyala, Iraq

Abstract

Background: *Blastocystis hominis* (*B.hominis*) is the most common intestinal parasite in humans and many other animals. Infections with the organism are spread worldwide and some of them have been asymptomatic, acute symptomatic and chronic.

Materials and Method: 100 blood samples were collected from patients with *B.hominis* which were reviewing to some Hospital and health center in Diyala province during the period from October 2018 to March 2019. To determine the extent of IgG antibody responses in serum patients infected with *B.hominis* for depending on the chromatic changes resulting from the association of antigens with antibodies.

Results: The results of the current study showed the percentage of infection among males was (56.25%) , which is higher than that of females (43.75%), and the age group (3-6) years among males showed the highest rate of infection (33.34%) and the lowest in age groups less from one year and (9-12) years groups at (11.11%). The rate of infection in the age groups(1-3) years and (6-9) years was (22.22%) and in the age group of (1-3) years was the highest incidence among female groups (42.86%), while the age group (3-6) years showed less than that (28.57%) and significant differences at the level of probability less than (0.05). The percentage of *B. hominis* infection increased among the rural population by (56.5%), male infected, (57.14%) female, while the proportion of males among the urban population was (44.44%) and females reached (42.86%), with a standard deviation of (1.600 ± 0.495) and (1.480 ±0.505) respectively.

Conclusions: The presence of immunoglobulin IgG in serum patients has been shown to stimulate the cellular immune response and be indicative of long-term immunity against pathogenic antigens.

Keywords: *Blastocytsis hominis , diagnosis, parasite, infection, patients.*

Introduction

B. hominis is one of the most common protozoa intestinal parasitic diseases worldwide and a common infected among humans and animals[1].It isolated from stool specimens appear as unicellul and it has multiple shapes, such as vacuolar, granular, and amoeboid [2]. It was considered as harmless yeast, but it is now getting acceptance as an agent of human intestinal disease especially under immunosuppressive conditions[3]. The extent research on *B. hominis* is transmission mechanisms, incubation period, epidemiology, and treatment options[4]. It's status as a true pathogen is controversial - while it has been found in patients with gastrointestinal symptoms with diarrhea or severe abdominal pain. Symptoms associated with human papillomavirus infection include: diarrhea, nausea, colic, abdominal distension, fever and chills. It is not proven to be the cause and many carriers are asymptomatic[5].

Corresponding author:
Muna Hameed Ahmed Alazzawi
Lecturer: Directorate of General for the Education of Diyala, Iraq

Diagnosis of *B.hominis* is used by clinical diagnosis of diarrheal symptoms and dehydration of the patient and laboratory methods. Direct microscopy of stool and cystic phase observation overlap with other causatives of diarrhea specially[6,7]. These data indicate that *B.hominis* induces as well as modulates the immune response in intestinal epithelial cells, and we conclude that different pathophysiological events may occur during *B. hominis* infection. [8]. The spread of disease is associated with poor hygiene, exposure to animals and the consumption of contaminated food or water [9] .

Iraq has seen widespread outbreaks of gastrointestinal diseases The Iraqi Ministry of Health and contributed many factors in the spread of the disease, including displacement of people to other places and water pollution.There are several studies that showed prevalence in different Iraqi governorates of the country. The rate of infection was recorded in 2013 (5.08%) for (31) children in Dohook in northern Iraq suffers from gastrointestinal symptoms[10]. 28 (4.1%) was infected with *B.hominis* from a total of 861 reviewers [11]. While in Muthana province in southern Iraq, the infection rate was 58 (45.67%) out of 127 patients infected with *B. Hominis* [12].The aim of the present study is to investigate the most important immunological changes in infected patients by measuring the IgG level, determining positive response rate, age and geuns, and setting up patients.

Materials and Method

Collection of samples

The present study collected 100 cases with *Blastocystis hominis* within the period from October 2018 to March 2019, of 50 male and 5o female, ranged in age less one year to 12 years . It has diagnosed the disease process and determined by dermatologists at some Hospital and health center in Diyala province and the injury was diagnosed based on the clinical symptoms and distinct phenotypic traits of infections.

Serological diagnosis

Serological diagnosis was performed by measuring the IgG level in the serum of patients with *B. hominis* to determine the response of the positive in the body of the infected patients. Blood samples were collected from patients. Was withdrawn 5 ml of venous blood for each of the infected persons , therapists and control the use of a syringe , put blood samples in test tubes and let the blood to clot at room temperature (20-25) minutes and then the separation of the serum Centrifuge device speeds of (3000) rpm and then it is kept freeze- preserved models when (-20˚C) until the subsequent immunological tests.

To find out the most important immunological changes associated with infection in patients with *B. hominis*, the study has included measuring levels of certain cellular dynamics in the serum of infected by and based on the principle of color change resulting from the correlation of quality IgG antibodies and measured color change resulting mediated.

Statistical Analysis

Statistical analysis of the results of the current study using the Statistical Package for Social Sciences conducted (SPSS) metadata. Test was used T-test, variance analysis ANOVA, Chi-square, percentage, standard deviation and standard error in the present study to find a moral differences between groups with infection *Blastocytis hominis* and there were significant differences ($P < 0.05$).

Results

The present study included 100 patients with *B. hominis*, 50 males and 50 females. The positive response rate of IgG for males was higher than that of females, which recorded positive response rate of (56.25%) at (1.820±0.388) .While the percentage of response to the antibiotic in the serum infected females (43.75%) at (1.860±0.351) . Hence, the statistical analysis of this increase indicates that there isn´t a significant difference of statistical significance at the level the probability is less than 0.05 as shown in Table 1.

Table: Effect of the sex factor on the positive serotonin of the IgG antibody of *Blastocyte hominis* for children covered in the infection

Groups	number	Positive response	Percentage%	Mean±SD	Stender Error(SE)	P-value
Boys	50	9	56.25	1.820±0.388	0.055	0.280
Girls	50	7	43.75	1.860±0.351	0.049	
Total	100	16	32%			

Table 2 shows that the injuries spread within a wide range of age groups. The age of those infected was less than 1 to 12 years. In males recorded age groups the (3-6) year age group had the highest IgG response rate at (33.34%) and followed by tow the age groups (1-3) years and (6-9) years (22.22%) and less than at (11.11%) in tow groups less one years and (9-12) years age groups. Compared to the female group, the (1-3) years age group had the highest incidence of IgG infection and response 42.86%.The lowest response among the age group of females was 0.00% in the age group less than one year, so that no percentage is recorded. This is due to the visitors who visit the health centers at the time of collecting samples. While the age group 3-6 years by 28.57% and less than the age groups (6-9) and (9-12) years by (14.29%). Differences between the proportions of m and their response to IgG were statistically significant at the probabilistic level the level the probability is less than 0.05 as shown in Table 2.

Table 2: Distribution of numbers and percentages of infection of *Blastocystis hominis* for the age of the infected and the extent of IgG response

Age groups in years	Number		% Number positive response				P-value
	Boys	Grils	Boys	%	Grils	%	
<1-	5	8	1	11.11	0	0.00	**0.028**
1-3	4	3	2	22.22	3	42.86	
3-6	7	10	3	33.34	2	28.57	
6-9	12	15	2	22.22	1	14.29	
9-12	22	14	1	11.11	1	14.29	
Total	50	50	9	100.00	7	100.00	

The patients' place of residence showed a clear difference between their urban and rural residence, but did not constitute a significant difference of statistical significance at the probability of p> 0.05.The prevalence of IgG immunoglobulin was significantly higher among urban than in rural areas as table3 showed . The proportion of males was 55.56% of respondents 5 patients of the total number is 20 cases, while females registered 57.143%, with 4 patients of the total number of 26 cases. The response rate in the urban group of infected males was 44.44% with 4 patients of 30 cases, less than 42.857% in urban areas, with 3 patients of the total 24 cases.

Table3: Effect of the living factor on the positive serotonin of the IgG antibody of *Blastocystis hominis* for children covered in the infection

Residence	Number tested		Number positive				P-value
	Boys	Girls	Boys	Percentage %	Girls	Percentage%	
Rural	20	26	5	55.56%	4	57.143	
Urban	30	24	4	44.44%	3	42.857	
Total	50	50	9	100.00%	7		**0.182**
mean±SD			1.600±0.495		1.480±0.505		
Stander Error(SE)			0.699		0.714		

Discussion

B. Hominis is a protozoan intestinal parasite disease that caused by the emergence of various symptoms, including diarrhea, nausea, abdominal cramps, bloating, excessive gas, and anal itching. The timescale of infection with the parasite can range from weeks to years [13].The present study showed an increase in IgG and positive response rate in males, with (56.25%) infection rate and (43.75%) female infection as in the table (2). This may be due to the number of hospital-reviewed cases and some male health centers more than females, and therefore dependent on individual immunity. This is consistent with the findings of Nayef *et al.*(2011)which indicated the percentage of male (23.99%) height on females (16.27%) and attributed the reasons for this to the above, attributed the reasons to as exposure to the infection increases with the top of nutrition and increased funding [14]. The percentage of infection between females and males does not make any significant difference at the probability level (0.05), which is consistent with the results of Mahmood and Khudher (2016) Indicating that there is no significant difference between the rates of infection of males and females and accounted for the percentage of infection *B. hominis* in Sammara city - Salah Al-Deen province was (9.09%) [15].

The study showed the effect of the age group in the incidence of *B. hominis*, whose age ranged between(> 1- 12) years with a significant difference of ($P < 0.05$). As it was the highest rate of infection in both genus and is attributed to the lack of development of the immune system in the younger age groups compared to the large age and this is consistent with what Salman (2015), who pointed to a significant difference at level ($P <0.05$) between different age groups ranging from 6 months to over 61 years out of a total of (177) patients with *B.hominis* [16]. The main cause of diarrhea is *B.Hominis* among other pathogens, as explained by al-Kaissi and Majdi (2009), the highest proportion of whom were 82 cases with *B. Hominis* isolated from 200 patients suffering from diarrhea by 41% [17]. Where the current study recorded the age group (3 – 9) years is the highest response rate positive for IgG among males infected with *B.hominis*, which amounted to (33.34 %) and the lowest was in the two age groups less than one year and (9-12) years is (11.11%). While the age group (1-3) years among females infected with the highest rate of (42.86%) and less than in the categories of age (6-9) years and (9-12) years is (14.29%), while the category less than a year does not register any case among females and this is due to patients reviewing hospitals and centers When collecting samples as showed in table (2) .

The current study indicates that the housing factor has a difference in the percentage of IgG positive response for patients with *B.hominis* for both sexes but does not register any significant difference at the probability level ($P > 0.05$). The positive response rate for patients with *B.hominis* who live in rural areas is higher than that of urban dwellers. This is due to the pollution of the environment and the environment of the rural inhabitants of *B.hominis*. They may be directly related to the reservoir stocks such as sheep, cattle, cats, dogs and other domestic animals. For rural males (55.56%) of respondents (5) patients of the total number is (20) cases, while females registered (57.143%), with (4) patients of the total number of (26) cases. The response rate in the urban group of infected males was (44.44%)

with (4) patients of (30) cases, less than (42.857%) in urban areas, with (3) patients of the total (24) cases as showed table (3).

Conclusions

The present study conclude that the disease's ability to stimulate the cellular immune response leading to the production of IgG that observed height in patients groups.

Conflict of Interest: None

Source of Funding: There is no funded support for the implementation of this work, while the active role of us as joint researchers in the design of the study and the collection of samples and analysis and preparation of the manuscript and financial and moral support.

Ethical Clearance: Blood sampling was performed from October 2018 to March 2019 with the consent of the reviewing patients to participate in this study.This study was completed.

References

1- El-Shazly, A. M., Abdel-Magied, A. A., El-Beshbishi, S. N., El-Nahas, H. A., Fouad, M. A., & Monib, M. S. ″*Blastocystis hominis* among symptomatic and asymptomatic individuals in Talkha Center, Dakahlia Governorate, Egypt″. Journal of the Egyptian Society of Parasitology, 35.2 (2005): 653-666.

2- Zierdt, C.H.″*Blastocystis hominis*: Past and future″. Clin Microbiol Rev; 4.1 (1991):61–79.

3- Lesh, F. A.″ Massive development of amebas in the large intestine″. Am. J. Trop. Med.Hyg., 24 (1975): 92-383.

4- Levine, N. D. ″Protozoan Parasites of Domestic Animals and Man″. Burgess Publishing Company, Minneapolis, MN, U.S.A.,54 (1961): 335-341.

5- Stenzel, D.J. & Boreham, P.F.L. ″*Blastocystis hominis* revisited″. Clin Microbiol Rev.,9.4 (1996):563-584.

6- Tan, K. S. ″*Blastocystis spp*. In N. A.; Khan (ed.), Emerging pmtoman pathogens″. Taylor and Francis, Oxford, United Kingdom. (2003a):342-358.

7- Tan, K. S. ″New insights on classification identification, and clinical relevance of Blastocystis spp″. Clin. Micmbiol. Rev., 21 (2008b): 639- 665.

8- Long, H.Y., Handschack, A., Konig, W. & Ambrosch, A.″*Blastocystis hominis* modulates immune responses and cytokine release in colonic epithelia cells″. Parasitol Res. Dec; 87.12 (2001): 1029-1030.

9- Yan, Y. S. S., Lai, R., Liao, H. Ye, J. & Li, X. ″Genetic variability of *Blastocystis hominis* isolates in China″. Parasitol Res; 99.5 (2006): 597–601.

10- Al-Saeed, A. T. M., IsmaeeQais, I. l. & Ahmed, G. F. ″*Blastocystis hominis* infections among patients in Duhok City / Kurdistan Region – IRAQ″. QM.J., 9.16 (2013): 161-170.

11- Al-Moussawi, A. M.″Prevalencoef Intestinal Parasite As among Rural population in Babylon Provinc″. Medical Jounral of Babylon; 2.4 (2005): 491-498.

12- Uobeed, A. H., Ali, G. B.& Mohammed, S. K.″Isolation and Identification of *Blastocystis homin*is isolated from Irritable Bowel Syndrome Patients using Phenotypic and Genotypic Methods″. International Journal of Scientific & Engineering Research; 6. 11 (2015): 1183-1190.

13- Fonte-Galindo, L., González-Rodríguez, Z., Fong-González, A., Méndez-Sutil, Y. & Moreira-Perdomo, Y. ″Evidencias y mecanismos de patogenicidad de *Blastocystis sp*″. Rev Cub Med Trop., 67. 1 (2015): 97-113.

14- Nayef, H. J., Majid, L. J.& Abdul Wahab, I. ″An epidemiological study of the prevalence of intestinal endometriosis in humans in Baghdad″. The University of Al-Nahrain J. 41.3 (2011): 57-36.

15- Mahmood, Omaima I. & Khudher Thu-Alfaqar A. Diagnostic and Epidemiological study of *Blastocystis hominis* in Samarra city, Iraq. Tikrit Journal of Pure Science 21. 6 (2016): 28-31.

16- Salman, Y. J. Detection of *Blastocystis hominis* among Peoples in Kirkuk Province Using ELISA and Direct MicroscopyInt.J.Curr.Microbiol.App. Sci., 4.10 (2015): 686-695.

17- Al-kaissi, E. & Al-Magdi, K. J. ″Pathogenicity of *Blastocystis Hominis* in Relation to Entropathogens in Gastroenteritis Cases in Baghdad″. EuroJournals Publishing, Inc,25.4 (2009): 606-610.

Epidemiological and Histopathological Study of Appendicitis in Karbala Province

Khetam L.Hussain[1], Aiyat hazem al-shugary[2], Eman Jabbar Rahim[3], Amear Ali Aenad[4], Sajida Jawad Mahdi[5], Abdullah ali Kate[6]

[1]Assistant Lecturer/ Alsafwa university college– medical laboratory techniques /Iraq, [2]Assistant Lecturer/ Alsafwa university college– medical laboratory techniques /Iraq. Assistant 3Lecturer/ Alsafwa university college– medical laboratory techniques /Iraq, [4]Assistant Lecturer/ Alsafwa university college– medical laboratory techniques /Iraq, [5]Researcher/ Alsafwa university college– medical laboratory techniques /Iraq, [6] Researcher/ Alsafwa university college– medical laboratory techniques /Iraq

Abstract

The current study included an epidemiological prevalence study of appendicitis in Karbala province, where complete data were collected for the year 2018 from Al Hussein medical Hospital. The results showed that the total number of appendicitis in Karbala province was (636) cases , divided in to (299) and (286) for male and female respectively, while the secondary removal cases was (69). The percentage of infection was 47.012%, 42.12% and 10.85% for male , female and secondary removal. The statistical analysis showed that there were significant differences under (P> 0.05) between percentage of infection in male and female (0.01). Also this study included histopathological examination of appendectomy specimen. After surgical removal of appendix, the specimen directly fixed with 10% formalin and then do tissue processing. The microscopic examination showed obstruction of the lumen and infiltration of inflammatory cell within lumina properia, muscularis and adventatia.the mucosal epithelial was destruction with ulceration, also there are extensive lymphoid hyperplasia.

Keywords*: appendicitis , prevalence, Karbala, gender, histopathological.*

Introduction

Appendicitis is inflammation of the vermiform appendix, this is a hollow organ located at the tip of the Cecum, usually in the right lower quadrant of the abdomen, however it can be located in almost any area of the abdomen depending on if there were any abnormal developmental issues (situs inversus totalis) or if there are any other concomitant conditions such as pregnancy or prior surgeries [(1).]

The appendix develops embryonically at the fifth week, during this time there is a movement of the midgut to the external umbilical cord with the eventual return to the abdomen and rotation of the Cecum, this results in the usual retrocecal location of the appendix [(2)].

Appendicitis is more common surgical emergencies, and it is one of the most common causes of abdominal pain [(3)] . The exact function of the appendix has been a debated topic, today it is accepted that this organ may have an immunoprotective function and acts as a lymphoid organ especially in the younger person, other theories contend that the appendix acts as a storage vessel for "good" colonic bacteria, still others argue that it is a mear developmental remnant and has no real function [(4)] .

Appendicitis is most common between the ages of 5 and 40 ,the median age is 28, risk factors include being male, higher household income and living in a rural area [(5)] .

In the United States, there were nearly 293,000 hospitalizations involving appendicitis in 2010 . Appendicitis is one of the most frequent diagnoses for emergency department visits resulting in hospitalization among children ages 5–17 years in the United States [(6)] .

Research aims:

1-study the epidemiological prevalence of appendicitis in karbala province.

2-study the histopathological changes occur in appendix tissues during acute appendicitis .

Research methodology and procedures:

A database of patients with appendicitis was collected from Al Hussein medical city in the Karbala city from January to December 2018. Also, samples were taken from the surgical theaters after being fixed directly with 10% formalin for tissue processing.

Results

1- Epidemiological study

All samples that collected in this study were under different condition, like; gender and age. The total number of appendicitis that show in this study in Karbala province was (636) cases divided in to (299 and (268) for male and female respectively, while the secondary removal cases was (69). The percentage of infection was 47.012%, 42.12% and 10.85% for male , female and secondary removal. The statistical analysis showed that there were significant differences under (P> 0.05) between percentage of infection in male and female (0.01). Table (1).

Table (1): Percentage of appendicitis according to gender:

Gender	Cases number	Percentage (%)
Male	299	47.012%
Female	268	42.12%
Secondary removal	69	10.85%
Total	636	100%
Chi-Square P-value	----	9.261 ** 0.0001
		** (P<0.01).

This study also show that the highly percentage of infection in both male and female was in age between 10-20 years (35,82% in female and 42,47% in male) , while the low percentage was in age between 60-70 years (1,11% in female and 0,66% in male) Table (2) and Table (3)

Table (2): percentage of appendicitis according to Female age:

Age / female	Cases number	Percentage (%)
$\geq 10\ year$	32	11,94%
$< 10 \geq 20\ year$	96	35,82%
$< 20 \geq 30\ year$	81	30,22%
$< 30 \geq 40\ year$	40	14,92%
$< 40 \geq 50\ year$	9	3,35%
$< 50 \geq 60\ year$	7	2,61%
$< 60 \geq 70\ year$	3	1,11%
Total	268	100%
Chi-Square P-value	--- 	10.026 ** 0.0001
** (P<0.01).		

Table (3): percentage of appendicitis according to Male age:

Age / Male	Cases number	Percentage (%)
$\geq 10\ year$	32	10,7%
$< 10 \geq 20\ year$	127	42,47%
$< 20 \geq 30\ year$	90	30,10%
$< 30 \geq 40\ year$	37	12,37%
$< 40 \geq 50\ year$	7	2,34%
$< 50 \geq 60\ year$	4	1,33%
$< 60 \geq 70\ year$	2	0,66%
Total	299	100%
Chi-Square P-value	---	10.934 ** 0.0001
** (P<0.01).		

2- Histopathological study:

In present study the histopathological changes observed in appendix were presence of extravascular polymorphs in the epithelium, lamina propria, or muscular layers was the main diagnostic feature of acute inflammation (Figure 1 B, C, D). The wall of appendix, was clearly visible and the mucosa was largely destroyed (figure 1(A, and D)), and there was extensive neutrophil infiltrate extending throughout the submucosa and into the muscularis externa. The was seen in all specimens. The glands of appendix was largely affected, and shows the mucosa glands was destroyed and pus present at the base of the gland and with only few remnant of glands in the section (Fig.21 B.

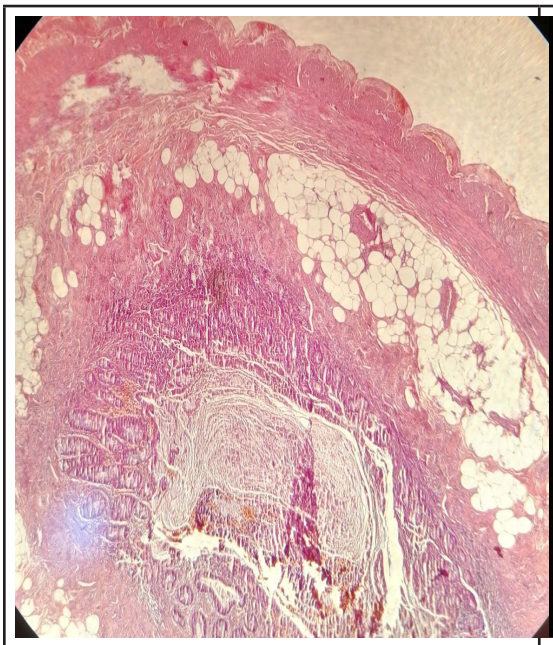


Figure (1) A:show lumen obstruction and mucosa destruction of appendix.

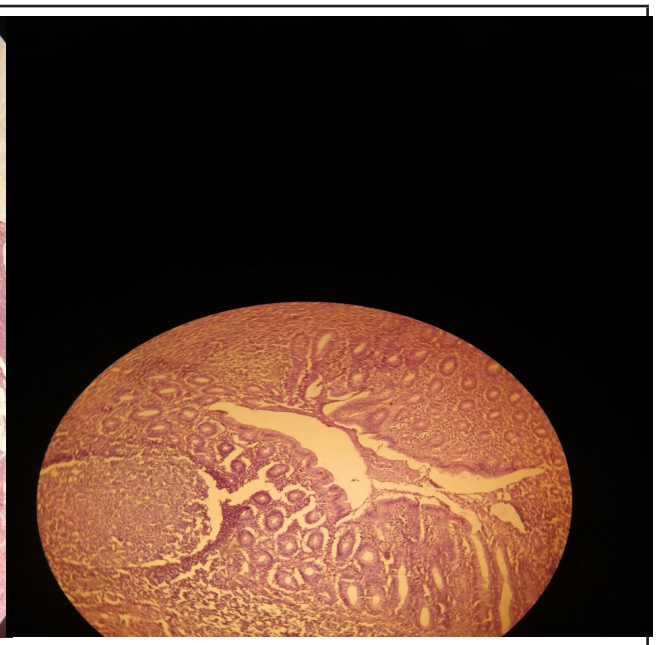


Figure (1) B: Show the lymphoid hyperplasia

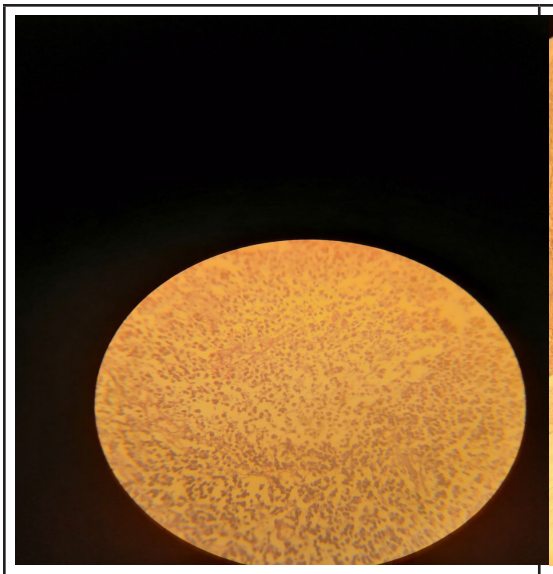


Figure (1) C:show infiltration of white blood cell (neutrophils).

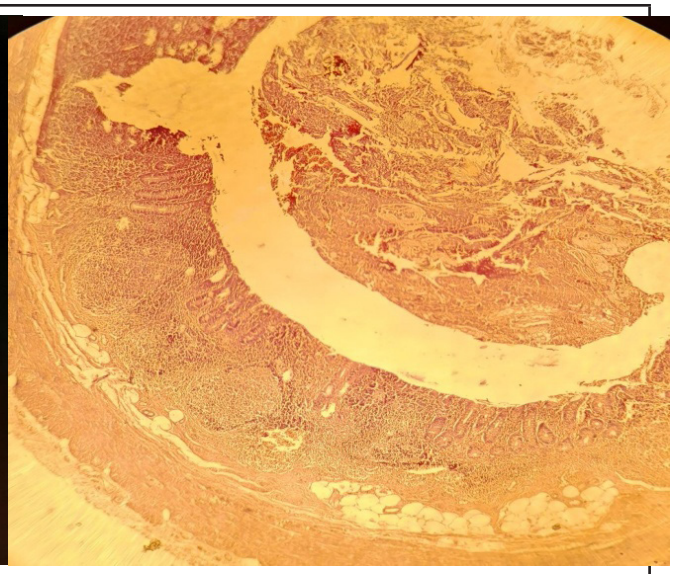


Figure (1) D:show obstruction of the lumen and infiltration of inflammatory cell within lumina properia , muscularis and adventatia

Discussion of Results

Our study was revealed increase incidence of appendicitis between males compare with females, that may be related with female sex hormones , which has been proposed because of lower incidence among women and incidence variations during the menstrual cycle [7] .

Anderson was found inverse relation between pregnancy and appendicitis, this suggests that pregnancy protects against appendicitis, especially in the third trimester. During pregnancy a range of physiological changes take place that may influence the pathogenesis of appendicitis [8]. Our finding was agree with [9] . who reported increase of incidence of appendicitis in males, this due to variation in body physiology between male to female.

In our study the highly percentage of infection is rang between age (10-20) years, that cited by [10]. who reported that incidence of appendicitis is generally a disease of young age. [11] was reported in his study the incidence outcome of appendicitis are related to age in young people. It has been suggested that the peak in the development of lymphoid tissue which occurs during adolescence leads to an increased liability of the appendix to obstruct, and so accounts for the high incidence of the disease.

the present histopathological study showed lymphoid hyperplasia with increase in lymph nodules diameter, these lead to obstruction. Our study was revealed increase in wall thickness of appendix and narrow lumen , with increase infiltration of white blood cells was very visible . this finding agree with [12] and [13] they recorded infiltrate of neutrophile in the muscularis mucosa and sub mucosa.

Obstruction of the appendiceal lumen seems to be essential for development of appendiceal infection. This obstruction occurs due to mucosal inflammation and lymphoid hyperplasia, once obstruction occurs, continued mucus secretion and increase intraluminal pressure, which obstructs lymphatic drainage and edema, mucosal ulceration and may cause venous obstruction, finally ischaemic necrosis of appendix wall produces gangrenous appendicitis.

Conclusions

1- Appendicitis is one of emergency disease.

2-The total cases in Karbala province was 636 in 2018 year which is divided to (299) and (286) for male and female respectively, while the secondary removal cases was (69).

3- The percentage of infection in male is higher than in female 47.012%, 42.12% and 10.85% for male , female and secondary removal.

4- Highly percentage of infection in both male and female was in age between 10-20 years (35,82% in female and 42,47% in male) , while the low percentage was in age between 60-70 years (1,11% in female and 0,66% in male).

5- The most histopathological lesions that found in appendectomy specimence was lumen obstruction and mucosa destruction of appendix, lymphoid hyperplasia, obstruction of the lumen and infiltration of inflammatory cell within lumina properia , muscularis and adventatia.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Vaos G, Dimopoulou A, Gkioka E, Zavras N .Immediate surgery or conservative treatment for complicated acute appendicitis in children. A meta-analysis. J. Pediatr: 2018,40(3).
2. Gignoux B, Blanchet MC, Lanz T, Vulliez A, Saffarini M, Bothorel H, Robert M, Frering V . Should ambulatory appendectomy become the standard treatment for acute appendicitis. World J Emerg Surg:2018, Pp:13:28.
3. Tamir IL, Bongard FS, Klein SR . Acute appendicitis in the pregnant patient. American journal of surgery:1990 ,571–6.
4. Eng KA, Abadeh A, Ligocki C, Lee YK, Moineddin R, Adams-Webber T, Schuh S, Doria AS . Acute Appendicitis: A Meta-Analysis of the Diagnostic Accuracy of US, CT, and MRI as Second-Line Imaging Tests after an Initial US. Radiology:2018 Pp:288(3):717-727.
5. Ellis H . "Acute appendicitis". British Journal of Hospital Medicine:2012,73 (3).
6. Barrett ML,Hines AL, Andrews RM . Healthcare Cost and Utilization Project Statistical . Agency for Healthcare Research and Quality:2016,10-20.
7. Elder S, Faragi D, Abrahamson J, Schein, M. The menstrual cycle and acute appendicitis. Eur J Surg:1995,pp:897-900.
8. Andersson R.Incidence of appendicitis during pregnancy. Inter J of epidemo:2001 ,pp:128-1285.
9. Oguntola AS, Adeoti ML, Oyemolade TA .appendicitis: Trend incidence,age,sex, and seasonal variation in south-western Nigeria. Annals of African medicine:2010 pp:213-217.
10. Al Omran M, Mandani M,Mcleod RS . Epidemologic features of acute appendicitis in Ontario. Surg, 2003: pp:46:263-8.
11. Lee JAH . The influence of sex and age on appendicitis in children and young adults. Gut: 1962,pp:3;80.
12. Aravindan K P . Eosinophil in acute appendicitis: possible significance. Indian J Patho Microbio:1979,40(4):491-8.
13. Ciani S. Chuaqui B . Histological features of resolving acute,noncomplicated phlegmonous appendicitis.Patho Res Pract:2000,196(2):89-93.

Molecular investigation of virulence factors genes in *streptococcus pyogenes* by PCR

Russell Issam AL-Daher[1], Maysa S. AL-Shukri[2], Hayam K . AL-Masoodi[3]

[1,2,3]Dept. of Microbiology, College of Medicine, University of Babylon, Iraq

Abstract

This study was designed to detect some genes associated with important virulence factors in *streptococcus pyogenes* . A total of 200 throat swabs were collected from patients suffering from pharyngitis from both sex and age from (1 - 15) years. carried out from ENT unit in Al-Hilla General Teaching Hospital and Al Noor Hospital during the period from January 2018 to December 2018 , Out of the 200 samples only 177 samples showed positive bacterial culture ,No growth was seen in other 23 samples ,The results indicate that the rate of *Streptococcus pyogenes* isolated from patient with pharyngitis is 30 (15%), others bacterial growth 147 (73%), and no growth 23 (12%).

Molecular detection of virulence factor genes was done like M protein (*emm*) gene , the result shows that these genes were detected in all isolates bacteria (100%) with molecular length (914 bp) , *SpeA* gene was carried by using specific primer and it was found that (3.3%) isolates give positive result for this gene with amplicon (576 bp) , *SpeB* gene the result shows that (100%) isolates contain the gene with molecular length (952 bp) , *SpeC* gene also study by using specific primer at molecular length (405 bp) and the result s show that (75%) give positive results to this gene , the *mac* gene in all *S.pyogenes* isolates the results show that (30%) give positive , results to this gene with molecular length (389 bp) , *scpA* gene in all isolates the result shows that 17 isolates (56.6%) give positive result to this gene with amplicon size (622 bp)

Keywords: *PCR ; streptococcus pyogenes ; virulence factors*

Introduction

Streptococcus pyogenes, commonly known as group A streptococcus (GAS) is a fermentative, facultative anaerobe, nonmotile, nonspore-forming gram-positive coccus, which occurs in chains or pairs, having a diameter of 0.5-1.0 μm. GAS are beta haemolytic streptococci. They require an enriched medium containing blood to grow [(1)]. The group A streptococci are fastidious organisms that have complex growth requirements. A highly nutritious growth medium that provides optimal growth GAS is generally grown on agar media supplemented with blood [(2)]. Pharyngitis, or commonly known as sore throat or strep throat, is the most common manifestation of infection with *Streptococcus pyogenes* (GAS) Infection with this bacterium is diagnosed in 20 to 40% of pharyngitis cases in children and in 5 to 15% in adults [(3)].M protein considered one of the most important virulence factors, the M protein promotes host interactions and adherence to human epithelial cells; specially helping bacteria to escape from host immune response by inhibition of phagocytosis [(4)].Other important virulence factors include the streptococcal superantigens (SAgs). SAgs are bacterial toxins which bind to major histocompatibility complex class II and T-cell receptors [(5)].

SpeB plays a role in the pathogenesis of *S. pyogenes* infections by Destruction of host defense system proteins and cleavage of GAS surface proteins may help the bacterium to escape immune clearance, invade the deeper tissues, and disseminate from the primary infection site [(6)].SPE-C was the first streptococcal SAg SPE-C binds to the polymorphic MHC class II β-chain with the formation of a tetravalent zinc complex that includes three residues within the C-terminal domain of SPE-C [(7)].Mac-2 is a related IgG endopeptidase that prevents the recognition of IgG bound to *S. pyogenes* by competitively blocking

Corresponding Author:
Russell Issam AL-Daher
Email : issamrussell@gmail.com

IgG from recognition by Fc receptors on host cells ,Given that IdeS/Mac have homologs across group A streptococcal strains [8]. Streptococcal C5a Protease ScpA, is a cell-bound peptidase anchored to the cell wall by sortase A ,The streptococcal C5a protease (SCP) is expressed on the surface of all serotypes of *S. pyogenes* and most human isolates of groups B, C, and G streptococci, where it specifically destroys C5a. The enzyme also binds fibronectin and functions as a low level invasion for *S. pyogenes*, group B streptococci and group G streptococci [9].

Materials & Method

This study included 200 patients (aged 1-15years) collected from throat swab who admitted to Al-Hilla Teaching Hospital, during a period extending from January 2018 to desember 2018. The specimens were collected from patients with pharyngitis to detect *Streptococcus pyogenes* by bacteriological analysis and vitek 2 system in a proper way to avoid any possible contamination

Detection of virulence Genes by PCR technique

DNA extraction and purification: This method was made according to the genomic DNA purification Kit supplemented by the manufacturing company Geneaid, (UK). The suspension containing DNA was stored at-20 C until used as a template for PCR. **Primer Sequences:** The primer sequences and PCR conditions that are used in the study[10].

Results and Discussion

Isolation of *streptococcus pyogenes* :

In this study, a total of 200 throat swab were collected from patients suffering from pharyngitis from both sex with age (1 - 15) years. carried out from ENT unit in Al-Hilla General Teaching Hospital and Al Noor Hospital during the period from January 2018 to december 2018 .

The results indicate that the rate of *Streptococcus pyogenes* isolated from a patient with pharyngitis is (15%), others bacterial growth (73%), and no growth (12%).as shown in table(1)

Table (1): Numbers and Percentages of *Streptococcus pyogenes*

Isolates From patients with pharyngitis

bacterial growth	Numbers of bacterial growth	Percentages %
Streptococcus pyogenes	30	15%
Other bacteria	147	73%
No growth	23	12%
Total	200	100%

The low percentage of *Streptococcus pyogenes* may be due to normal flora that found in the pharynx and other bacteria that cause secondary infection , there compete pathogenic bacteria in nutrient in culture media

No growth was seen in other 23 samples which indicate the presence of other microorganisms that may be cultured with difficulties such as viruses , fungi and other agents or because of the misuse of antibiotics that cause the disappearance of the bacteria. Antibiotic treatment is recognized as an effective means to reduce transmission of the organism particularly for respiratory and cutaneous infections [11].

Genetic detection of Virulence factors of *Streptococcus pyogenes* by PCR

Molecular detection of (*emm* gene)

By using specific two primers for detection of M protein (*emm*) gene , the result shows that these genes were detected in all isolates bacteria (100%) with molecular length (914 bp) when compared with allelic ladder as shown in figure (1)

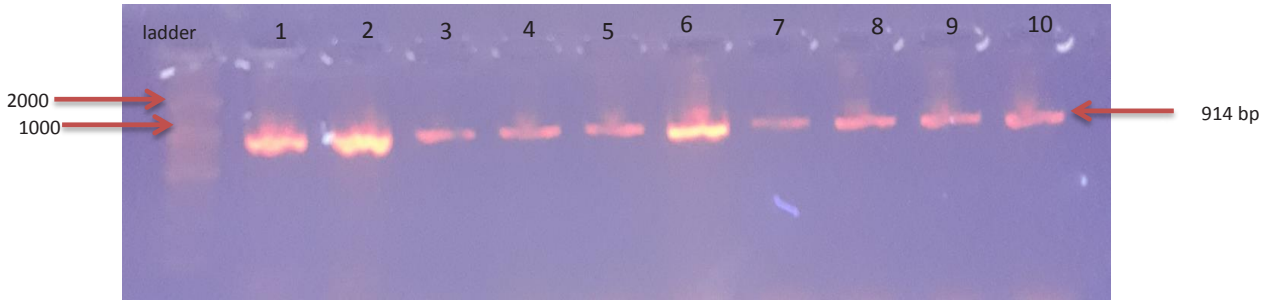


Figure (1) agarose gel electrophoresis at 70 volts for 50 min. for *emm* gene in *S. pyogenes*. PCR product visualized under U.V light at 320nm. After staining with ethidium bromide. L: Ladder with 2000 bp. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 were positive for this gene with amplicon size 914 bp.

Khosravi *et al*, (2016) found that the *emm* gene detects in all isolates of GAS isolates isolated from throat samples in children with sore throat , and found that the types of *emm* gene different according to the types of diseases[12].

Molecular detections of pyrogenic exotoxins (*SpeB* ,*SpeC*)

In this study two primers were used to detect the SpeB gene and the result show that (100%) isolates contain the gene with molecular length (952 bp) when compared with allelic ladder as shown in figure (2)

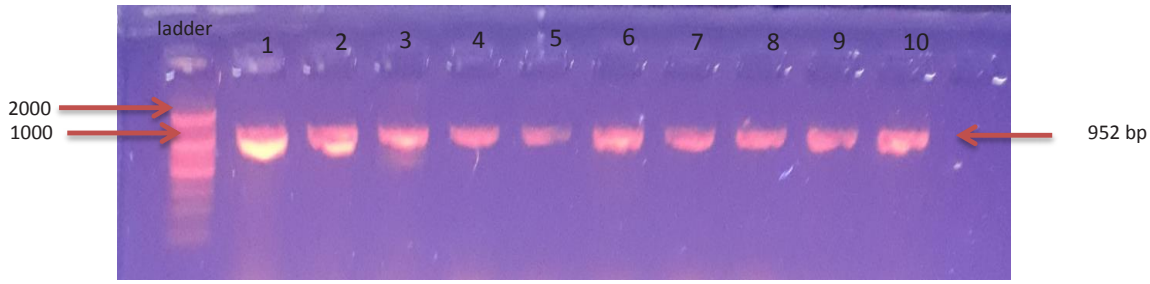


Figure (2) agarose gel electrophoresis at 70 volts for 50 min. for *speB* gene in *S. pyogenes*. PCR product visualized under U.V light at 320nm. After staining with ethidium bromide. L: Ladder with 2000 bp. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 were positive for this gene with amplicon size 952 bp.

Hytonen *et al ,(*2001) found that the *SpeB* gene is carried by all strains of *S.pyogenes* , but the degree of expression varies from strain to strain , and *SpeB* has been considered to produce only in a secreted form . the expression of *SpeB* is controlled by the multiple gene activator *mga* [13].Also in this study the *SpeC* gene also study by using specific primer at molecular length (405 bp) and the result s show that (75%) give positive results to this gene when compared with allelic ladder as shown in figure (3)

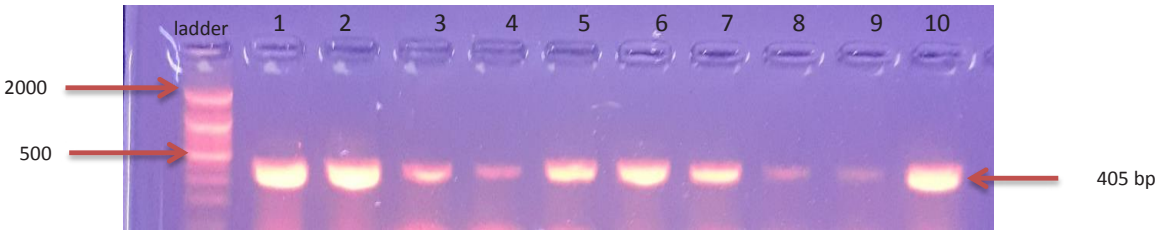


Figure (3) agarose gel electrophoresis at 70 volts for 50 min. for *speC* gene in *S. pyogenes*. PCR product visualized under U.V light at 320nm. After staining with ethidium bromide. L: Ladder with 2000 bp. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 were positive for this gene with amplicon size 405 bp.

In this result it was found that *SpeB* is more prevalence than other gene *SpeC* this may due to that this gene coded by chromosome and *SpeB* is the key virulence factors in GAS pathogenesis .

Also the distribution in the prevalence these genes may attributed to site of infection or may the isolates contain other genes responsible for exotoxin , which it was found that there are (11) gene responsible for *Streptococcus pyogenes* exotoxin

Complement membrane attack complex (Mac) gene detected by PCR

Specific primer was used to amplify the *mac* gene in all *S.pyogenes* isolates the results show that (30%) give positive , results to this gene with molecular length (389 bp) when compared with an allelic ladder as shown in figure (4)

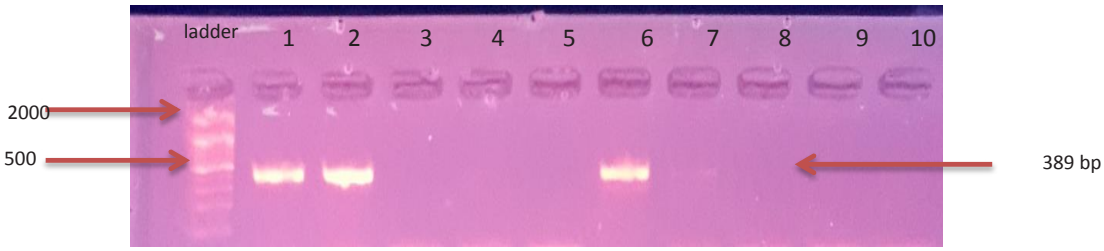


Figure (4) agarose gel electrophoresis at 70 volts for 50 min. for *mac* gene in *S. pyogenes*. PCR product visualized under U.V light at 320nm. After staining with ethidium bromide. L: Ladder with 2000 bp. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 were positive for this gene with amplicon size 389 bp.

The different the percentage of this gene may be due to the bacterial contain other gene encoded for complement factor degradation other than mac like Endos and C5a peptidase .

Molecular detection of scpA (streptococcal C5a peptidase) by PCR .

DNA was extracted from (30) *streptococcus pyogenes* isolates , PCR was carried outusing these DNA from the amplification of specific primer (scpA) after the gel electrophoresis , the result shows that 17 isolates (56.6%) give positive result to this gene with amplicon size (622) when compared with allelic ladder as shown in figure (5)

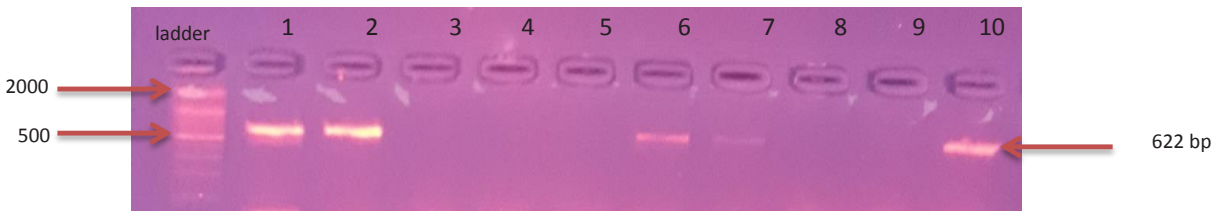


Figure (5) agarose gel electrophoresis at 70 volts for 50 min. for *scpA* gene in *S. pyogenes*. PCR product visualized under U.V light at 320nm. After staining with ethidium bromide. L: Ladder with 2000 bp. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 were positive for this gene with amplicon size 622 bp.

scpA decreases the rate of GAS clearance by inhibiting chemotactic recruitment of phagocytic cells to the site of infection , also shown to promote Fn independent GAS invasion of the human epithelial cell [(14)]. The negative results may belong to that the isolates contain another gene responsible for protease gene like (speB and SPYCEP) or gene is non-functional gene.

Conclusion

Humans are the only reservoir for GAS. It is most common among children 5 through 15 years of age ,presence of superantigen genes within the S. pyogenes genome suggests that they do play a significant role in S. pyogenes disease , high prevalence of *emm ,speB, sdaB* gene in *streptococcus pyogenes* isolates .

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Amano A, Nakagawa I .Autophagy in Innate Immunity against Intracellular Bacteria, *The Journal of Biochemistry*. 2006;161–166
2. Shulman ST, Bisno AL, Clegg HW. Clinical practice guideline for the diagnosis and management of group A streptococcal pharyngitis update by the Infectious Diseases Society of America. *Clin Infect Dis*. 2012;55(10):e86–e102.
3. Shaikh N, Swaminathan N, Hooper EG. Accuracy and precision of the signs and symptoms of streptococcal pharyngitis in children: a systematic review. *J Pediatr*. 2012;160(3):487–493.
4. Friães A, Pinto FR, Silva-Costa C, Ramirez M, Melo-Cristino J, Portuguese Group for the Study of Streptococcal Infections. Group A streptococci clones associated with invasive infections and pharyngitis in Portugal present differences in emm types, superantigen gene content and antimicrobial resistance. BMC microbiology 2012;1186/1471-2180-12-280.
5. Lintges M, van der Linden M, Hilgers RD, Arlt S, Al-Lahham A, Reinert RR, et al. Superantigen genes are more important than the emm type for the invasiveness of group A Streptococcus infection. J Infect Dis. 2010;202(1):20–8.
6. Walker M J, Barnett TC, McArthur J D. Disease manifestations and pathogenic mechanisms of group A streptococcus. Clinical Microbiology Reviews.2014; 27(2):264–301.
7. Fernandez M M, Guan R, Swaminathan C P. Crystal structure of staphylococcal enterotoxin I (SEI) in complex with a human major histocompatibility complex class II molecule. *J. Biol. Chem*.2006; 281, 25356–25364.
8. Okumura CY, Anderson EL, Döhrmann S. IgG protease Mac/IdeS is not essential for phagocyte resistanceor mouse virulence of M1T1 group AStreptococcus. mBio . 2013; 4(4):e00499-13.
9. Wei Z,Fu Z, ChenY . *Streptococcus equi ssp. zooepidemicus* C5a peptidase, a putative invasin, induces a protective immune response in mice Research in Veterinary Science .2013; 444-450
10. Borek AL, Obszanska K, Hryniewicz W..Detection of Streptococcus pyogenes virulence factors by multiplex PCR . Virulence .2012;3:6, 529–533.
11. Steer AC, Carapetis JR, Dale JB, Fraser JD, Good MF, Guilherme L, Moreland NJ, Mulholland EK, Schodel F, Smeesters PR. Status of research and development of vaccines for Streptococcus pyogenes. Vaccine .2016; 34:2953–2958.
12. Khosravi, A.; Ebrahimifard, N. ; Shamsizadeh , A. and Shoja ,S . (2016).Isolation of Streptococcus pyogenes from children with pharyngitis and emm type analysis J. ournal of the Chinese Medical Association . 276-280.
13. Hytonen J, Haataja S, Gerlach D, Podbielski A. The SpeB virulence factor of Streptococcus pyogenes, a multifunctional secreted and cell surface moleculewith strepadhesin, laminin-binding and cysteineprotease activity Molecular Microbiology .2001;39(2), 512-519.
14. Kagawa TF, Toole PW, Cooney JC. SpeB-Spi: anovel protease-inhibitor pair from *Streptococcus pyogenes* . Mol.Microbiol.2005; *57* , 650 – 666.

Iron Deficiency among Patients with Febrile Seizures in Al Ramadi Maternity and Children teaching Hospital, Western Iraq

Sahar j. AL- Hiali

Assistant Professor. Department of Pediatrics, college of Medicine, Ibn Sina University of Medical and Pharmaceutical Sciences, Baghdad, Iraq

Abstruct

Background : Febrile seizures are the most predominant neurological disorders in children between 6 months-5 years, at the same time iron deficiency anemia is one of nutritional insult that implicated as risk factor for occurrence of febrile seizures.

Objectives: to estimate the role of iron deficiency as a risk factor for febrile seizures in children aged 6 months -5 years, western Iraq.

Patients and method:- A case control study has been conducted in AL Ramadi maternity and children teaching hospital during January to October of 2016. All children with history of febrile seizures aged 6 months-5years were involved as cases group, others with febrile illness and no seizures were considered as control group. Data from 58 child of each group were collected. The data include age, sex ,temperature, causes of febrile illness, Hb ,hematocrit and s. ferritin levels. The data were subjected to statistical analysis run under IBM SPSS ver. 23.

Results:- Means age ± SD were 27.48±14.83 & 23.24±14.37 months in cases and control groups respectively with no significant difference between them. Most of the children (65.52%) of those with febrile seizures were less than 3 years old. Mean temperature of cases group (38.86^0 C) was found to be significantly greater than that of the control group (38.52^0 C) at p-value <0.01. Mean Hb level ,PCV%, s. ferritin level were significantly lower in cases group than in control group. Proportion of iron deficiency anemia was significantly higher in febrile seizure group (65.52%) than in control group (31.03%) at p-value< 0.01**.**

Conclusions: Occurrence of IDA in cases group is found to be more than 2 times of that in the control group. Early detection and proper treatment of IDA can play a prominent role in limitation the prevalence of febrile seizures among children below 5 years.

Key words*: febrile seizures, IDA, western Iraq*

Introduction

Febrile seizures (FSs) is the most predominant neurological disorders in childhood with a multifactorial inheritance that occur in 2-5% of children in united states,5-10% in India and up to 14% in Guam[1-3].

Corresponding author
Sahar j. AL- Hiali
Email: saharjabbarkadhum@gmail.com

FSs described as a seizures that come in association with high grade fever of 38 centigrade or more without brain infection or electrolytes disturbances involving age groups ranged from 6months-5 years with peak age of occurrence of 18-24 months[4,5].

Iron deficiency anemia (IDA) is another well pronounced easily correctable nutritional problem that can affect the intellectual development and occur predominately in a nearly similar age interval of FSs in young children 6months -24 months[6,7].

In general, anemia was noted in a proportion 46-66% of children younger than 4years of in developing countries and approximately half of them were iron deficient[8] .

Indeed, iron is one of the micronutrient that have an important role in neuronal development (neurogenesis), maturation of myelin, energy and neurotransmitters metabolism [9,10] as well as formation of hemoglobin.

Thus abnormal neurotransmitters functions as a sequence of iron deficiency ,may in turn contribute to alteration of seizure threshold and initiation of febrile seizures in young children[7].

So if we take into account the same age prevalence of IDA& FSs and the effect of iron deficiency on brain function ,as well as the value of Hb in carrying O2 to brain ,and since presence of fever can exaggerate the symptoms that result from effect of IDA on brain ,so relation between FSs &IDA is probable .

Based on all the above considerations, and since no study was conducted in al Ramadi province western Iraq, about such problem ,so our study was conducted to estimate the role of iron deficiency as a risk factor for febrile seizures among children from 6months-5 years ,western Iraq.

Patients and Methods

A case control study has been conducted in AL Ramadi maternity and children teaching hospital, western Iraq during the period January to October of 2016.The study approved by the ethics committee of AL Anbar University, Medical College. All children who admitted with history of febrile seizure and their age ranged from 6 months-5 years were involved in the study as a febrile seizure group (FSG). The control group collected randomly from children between 6 months -5 years who they admitted for same hospital during study period with febrile illness & no seizures (non-seizure group, NSG).

Detailed history and physical examination were done for them. Simple febrile seizure defined as single seizure that associated with peak of fever, last less than 15 minutes, once per day and generalized features, complex febrile seizures defined as seizure last more than 15 minutes, frequent per day or had focal features [11]. All patients with history of CNS infection and history of delayed developmental milestone were excluded .

Information that collected from studied groups include age, sex , degree of temperature at admission, causes of febrile illness (respiratory tract infection, gastroenteritis, otitis media, tonsillitis, others), nature of seizures, duration and frequency of seizure were all recorded for every patient. Five mls of blood was aspirated to assess Hb, hematocrit (PCV) and s. ferritin values in studied groups(ferritin Accu Bind ,ELISA Microwells ,USA).

IDA defined as Hb level less than 11gm/dl. PCV less than 30%, s. ferritin less than 12ng/ml [6,12].

The data were statistically analyzed using IBM SPSS v. 23.0, p-value < 0.05 was considered significant.

Results

One hundred sixteen children aged 6 months-5years were included in the study during the period mentioned previously. Patients were classified into two groups each of 58 patients. The cases group involved 34 males and 24 females which indicates a male to female ration of 1.42:1, on the other hand, the control group consist of 36 males and 22 females with a male to female ratio of 1.64:1.

Regarding FSG,48 (82.7%) of them had simple type febrile seizures, and the remaining cases had complex type(17.3%). Most of patients with febrile seizures 38(65.52%) were below age of 3 years. Out of this group 22(37.93%) their age ranged from 12-24 months. Only four patients (6.9%) were found in the age group 6-12 months (Table 1).

The means age were 27.48±14.83 and 23.24±14.37 in FSG & NSG respectively with no significant difference between them (p =0.120), (Table 2).

The Chi-square test revealed no significant association between gender and groups of the study (Chi-square=0.144, p-value=0.7). Percentages of gender groups were compared to each other in both groups and found to be not significantly different (table 2). Mean temperature at admission was significantly higher in FSG than NSG ,(p= 0.001).

Respiratory tract infection was significantly the most predominant cause of febrile illness in FSG 30 (51.7%) as compared to NSG 10 (17.2%), whereas gastroenteritis was significantly lower in FSG 20 (34.5%) as compared to NSG 38 (65.5%) (Table 2).

Means Hb level , PCV% & s. ferritin levels were significantly lower in FSG than in NSG, p values were all lower than 0.05.

The proportion of IDA among FSG was significantly higher than that of NSG , 38 (65.52%) vs. 18 (31.03%) , p-value=0.0003.

Table1: Distribution of patients according to age, gender and case-control groups.

Age (Months)	**FSG (n= 58)**		**NSG (n=58(**	
	Gender		**Gender**	
	Males N(%)	**Females N(%)**	**Males N(%)**	**Females N(%)**
6-<12	0(0.0)	4(16.67)	10(27.78)	6(27.27)
12-<24	14(41.18)	8(33.33)	12(33.33)	8(36.36)
24-<36	4(11.76)	8(33.33)	2(5.56)	6(27.27)
36-<48	6(17.65)	4(16.67)	0(0.0)	2(9.09)
48-60	10(29.41)	0(0.0)	12(33.33)	0(0.0)
Total	34(100)	24(100)	36(100)	22(100)

Table 2: Demographic Characteristic and Hematological Findings Of Febrile Seizure Group and Non-Febrile Seizure Groups

		FSG(cases) (N=58)	**NSG(Controls) (N=58)**	**P value**
Age, months(mean		27.48±14.83	23.24±14.37	0.120
Gender	Male N(%)	34(59)	36(62)	0.74
	Female N(%)	24(41)	22(38)	0.74
Temperature c0(mean ±SD)		38.86 ± 0.544	38.52 ± 0.580	0.001 *
Causes of febrile illness N(%)	Respiratory tract infection	30(51.7)	10(17.2)	0.0002*
	Gastroenteritis	20(34.5)	38(65.5)	0.011*
	Otitis media	4(6.9)	4(6.9)	1.000
	others	6(10.3)	6(10.3)	1.000
§ Hb g/dl(meanSD)		10.54±0.83	11.16±1.12	0.001*
§§ Pcv%(meanSD)		30.72±1.58	34.50±3.63	0.000*
s. ferritin ng/ml (mean)		20.97±11.44	43.59±26.15	0.000*
IDA N(%)		38(65.52)	18(31.03)	0.0003*

§ Hemoglobin, §§ Packed cell volume, * Significant difference

Discussion

Febrile seizures are the most common neurological disorder that occurs in children less than 5 years old without central nervous system infection or electrolyte disturbances.

In the current study FSs were pronounced predominately in children below age of 3 years (65.52%), mainly among 12-24 months age group which is in agreement with other studies[13,14].

The mean age of onset of FSs was 27.48 months which is nearly similar to others [4,5,15,16].

As described by other researchers ([13,17]) majority of patients with FSs had simple type of seizure (82.7%) and the remaining had complex type. The association between FSG, NSG and gender groups was statistically insignificant which is comparable with results of other reports[18,19].

Moreover, Like other reports[15,20,21],this study revealed a significant high peak temperature at admission in FSG than NSG. It was generally reported that fever is one the risk factors implicated in occurrence of FSs that may aggravate the worse effect of lack of iron on brain function which may precipitate seizure attack.[17,22]

Regarding causes of febrile illness, respiratory tract infection was the predominant cause of febrile illness in FSG which is found to be significantly higher than that of the NSG and that is in agreement with other studies [23-25].

On the other hand gastroenteritis was significantly lower in FSG than NSG which supports the suggestion of its protective effect against FSs as reported by other studies [5,26].

Several previous publications from different countries worldwide clearly demonstrated the existence of association between IDA and FSs and they considered IDA as an important risk factor for occurrence of FSs[9,27,28].

This study carried out in the Western region of Iraq in order to cast light on some of the important concepts associated with main issue of the study. Accordingly, this study showed that there are statistically significant reduction in means Hb level ,PCV %, s. ferritin level of (FSG) compared to (NSG), in addition the proportion of IDA in FSG was more twice that in the NSG (table 2). This results is in agreement with Daoud A S etal's report, from Jordan, 2002[29] who study the association between IDA & s. ferritin ,they thought that lack of iron could be possible cause of FSs. as they found a significant low mean s. ferritin levels and higher percentage of cases with low serum ferritin 30ug/dl among FSG than controls ,29±21mcg/L vs. 53.3±37.9mcg/L ,(p 0.000).

On other hand, Kumari et al, 2012 [30] used univariate and multivariate analysis for the data collected from the same purposes of this study, they observed a significant higher proportion of iron deficiency in FSG than controls, 63.6% vs.24.7% ,p=0.001 which is complies with the results of the current study. The same conclusion reported by Momen A et al., 2010 from Iran[18],whom they confirm the existence of positive relation between febrile seizure and IDA.

Habibian N ,et al., 2014[31] conducted a meta-analysis study and observed that IDA was moderately increased the chance of FSs in children especially in the regions of low or moderate percentage of IDA.

Moreover, similar significant association between IDA &FSs was pronounced by El-Shafie et al., [32], 2017 whom conducted a prospective case control study of 60 cases aged 6 months -5 years from Egyptian children, they found that 21 (52.5%) of cases had IDA compared to 4 (20%) of controls p 0.05.

However, other studies had proved otherwise and denied any association between IDA and FSs and thus did not agree with results of this study. Amirsalari etal[33] Omen etal [34] and Abaskhanian A etal[35] deduced a lack relationship between IDA and FSs.

Furthermore, Derakhshanfar H, etal[5] suggested that IDA was less common in febrile seizure patients than healthy children. Whereas Talebian A ,etal [36] in a study involved 120 children aged less than 5 years, reported that IDA was more common in controls than FSG and concluded that IDA was not a risk factor but rather a protective factor against FSs.

Bidabadi E and Mashouf M,[15] in a case control study of 200 cases with febrile seizures and 200 controls aged 6 months-5 years, found that IDA was less frequent in patients with FSs than controls with no statistical difference between the two groups, but the protective effect of iron deficiency against FSs was not proved (odd ratio 1.175).

Indeed, these variation in results of these reports could be related to the variations in studies designs, different samples sizes and age groups involved, different measures used for diagnosis of IDA, nutritional status and prevalence of IDA among children involved in these studies from different regions. Unfortunately, no previous study was carried out in al Ramadi province, Western Iraq, regarding prevalence of IDA among children aged less than 5 years. Based on the results of this study, it can be concluded that IDA is about two times more predominant in FSs patients than in other patients with no FSs, and Iron deficiency is well recognized as a risk factor in children aged less than 5 year. In this context, early detection with proper correction of IDA will be effective in reducing rates of FSs among those children. Large sized prospective study is necessary to assess prevalence of IDA in children less than 5 years as well as to help evaluating nutritional problems that may contribute positively to the IDA.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Abou-Khalil BW, Gallagher M J, Macdonald R L. Epilepsies. In: Daroff RB, Jankovic J, Mazziotta JC, Pomeroy SL, eds. Bradley's Neurology In Clinical Practice. 7th ed. Philadelphia, PA: Elsevier; 2016:chap 101.
2. Shinnar S. Febrile Seizure .In: Swaiman KF ,Ashwal S ,Ferriero DM , editors. *Pediatric neurology principles& practice* .4th edition ,vol1.philadelphia:Lippincott Williams &Wilkins,2006.P.1079-89.
3. PaulSP,Blaikley S, Chinthapalli R. Clinical update :Febrile convulsion in childhood. *Community Pract*.2012;85:36-8.
4. Rosman NP. Evaluation of the child who convulses with fever .*Paediatr Drugs* . 2003;5:457-61.
5. Shinnar and T.A. Glauser .Febrile seizures . *J Child Neurol* .2002;17suppl1:s44-52.
6. World Health Organization .Iron Deficiency Anemia Assessment ,prevention and control. A guide for program Manager .*WHO/NHD*/013:Geneva:2001.
7. Beard JL. Iron deficiency alters brain development and functioning . .*Jnutr*.2003;133:1468-72.
8. Stoltzfus R. Defining iron deficiency anemia in public health terms :a time for reflection .*J Nutr* 2001;131(2s-2):5655-75.
9. Sherjil A, Us Saeed Z , Shehzad S, Amjad R. Iron deficiency anemia –a risk factor for febrile seizures in children .*J Ayub Med Coll Abbottabid.* 2010;22(3) :71-3.
10. Rouault TA ,Coopermans .brain iron metabolism .*Semin Pediatr Neurol* .2006;13:142-8.
11. Mikati M.A. Febrile seizures .In Stanton B.F, Schor N.F. Behrman R.E, Geme J.W.S. *Nelson Textbook of Pediatrics* 19th edition .Saunders Elsevier 2011;2017-2018.
12. DegrucchG C .Clinical Hematology in Medical practice ,5th.Victoria :Black well science Ltd;2004.
13. Aliabad GM ,Khajeh A , Fayyazi A, Safdari L. Clinical, epidemiological and laboratory characteristics of patients with febrile convulsion .*Journal of comprehensive Pediatrics* .2013;3(4):134-7.
14. Shrestha D, Dhakal AK, Shakya H, Shakya A, Shah S C, Mehata S .Clinical characteristics of children with febrile seizure. *JNepal Health Res Counc*.2014;12(28): 162-6.
15. Bidabadi E , Mashouf M. Association between iron deficiency anemia and first febrile convulsion :A case control study.*Seizure*2009;18:347-51.
16. MajumdharR,HaricharanKR , Venkatamurthy M .Iron deficiency as a risk factor for first febrile seizure. *Journal of Evolution of med and dental Sciences* 2013;2(21)3834-3840.
17. Ghasemi,Valizaheh F, Taee N. Iron deficiency anemia in children with febrile seizure: A case –control study .*Iran J child Neurol*.2014;8(2):38-44.
18. Momen AA, Nikfar R, Karimi B .Evaluation of Iron status in 9 month to 5 year old children with Febrile seizures : A Case –Control Study in the south west of Iran .*Iran J Child Neurol* .2010;4(2):45-50.
19. Modarisi M ,Mahmoudian T ,Yayhini O, Kelishadi R ,Golestani H ,Tavasoli A ,etal.Is Iron insufficiency associated with Febrile seizure ? Experience in an Iranian Hospital .*J compr ped*2012;3(1):21-4.
20. Ala B ,Nathalie G ,Isabel F,etal .Genetic influence

on the clinical characteristics and outcome of febrile seizures-A retrospective study .*European Journal of Pediatric Neurology*. 2005;9:339-345.

21. Derak hshanfar H ,Abask hanian A ,Ali mohammadi H, Modanlookordi M. Association between iron deficiency anemia and febrile seizure in children .Med *Glas (zenicko.)*2012;9(2):239-42.
22. Glader B. Anemia of Inadequate production .In. Kliegman R, Behrman R, Jenson H,S tanton B (Eds) .*Nelson Textbook of pediatrics* .18th ed .philadelphia ,USA Saunders Elsevier;2007.p.2014-17.
23. WinklerAS,TluwayA, Schmutzhard E. Febrile seizures in rural Tanzania :hospital-based incidence and clinical characteristics .*JTrop Pediatr*.2013;59(4):298-304.
24. ShresthaSK.Role of CSF analysis for the first episode of febrile seizure among children between six months to five years ago .*J Nepal pediatr SOC*.2010;3092):90-3.
25. Kaputu Kalala Malu C, Mafutu Musalu E ,Dubru J M ,Leroy P,Tomat A M , Misson JP .Epidemiology and characteristics of febrile seizures in children .*Rev Med Liege*.2013;68(4):180-5.
26. Lee E H, Chung S. A comparative study of febrile and afebrile seizures associated with mild gastroenteritis .*Brain Dev*.2013;35636-40.
27. Hartfield DS ,Tan J ,Yager JY ,Rosychuk R J ,Spandy D ,Haines C ,Craig WR. The association between iron deficiency and febrile seizures in childhood .*Clin Pediatr (Phila*).2009;48:420-426.
28. Naveed-Ur-Rehman ,Billo AG. Association between iron deficiency anemia and febrile seizures .*JColl phys surg pak*.2005;15:338-340.
29. Daoud AS , Batieha A , Abu-Ehteish F , Gharaibeh N, Ajlouni S, Hijazi S. Iron status :a possible risk factor for the first febrile seizur.*Epilepsia*.2002;43:740-3.
30. KumariPL, Nair MK, Nair SM, Kailas L, Geetha S. Iron deficiency as a risk factor for simple febrile seizures –a case control study .*Indian Pediatr*.201249(1):17-9.
31. Habibian N, Alipour A ,Rezaia Zaheh A. Association between Iron Deficiency Anemia and Febrile Convulsion In3 –to 60 months old children: A systemic Review and Meta-Analysis .*IJMS*.2014;39(6):496-505.
32. El-Shafie AM , Abou EI-Nour ESS , EI-Hawy MA .Study of iron deficiency anemia in children with Febrile seizure. *Menoufia Med Journal*. 2017;30(1):209-212.
33. Amirsalari S, Keihani Doust Z T , Ahmadi M, Sabouri A , Kavemanesh Z , Afsharpeyman S, etal .Relationship between iron deficiency anemia and febrile seizures .*Iran J Child Neurology*.2010:4(1):27-30.
34. OmenM, Hakim Zadeh M.Case-control study of th relationship between anemia and febrile convulsion in children between 9 months and 5 years of age. *Ahuaz J Uni Med Sci* .2003;1:50-4.
35. Abaskhanian A, Vahid shahi K , Parvinnejad N. The Association between iron deficiency and the first episode of febrile seizure .*J Bablo Univ M ed Sci.*2009;11(3):32-36.
36. Talebian A, Momtaz manesh N. Febrile seizures and Anemia .*Iran J Child Neurol*.2007:2(1):31-3.

Antibiotic Susceptibility of Bacteria Isolated From Under Nails

Zahraa AL-Taee[1], Sura Ihsan Abed Jabuk[1], Raflaa S.H. Hussian[1], Zainab H. Kareem[2], Yasir H. AL-Mawlah[1]

[1]Lecturure Dr., [2]Assistant Lecturer, University of Babylon, College of Science, Department of Biology, Hilla, Iraq

Abstract

This study was designed for isolation and diagnosis of bacteria from under long dinger nails of a wide population of student (kindergarten, primary schools, elementary schools and university students) and study the antibiotic sensitivity for isolated bacteria.

From 100 sample the bacterial isolate were *Staphylococcus* sp.(56 isolate), *Bacillus* sp.(1 isolate), *Streptococcus* sp. (1 isolate), *Escherichia coli* (40 isolate), *Salmonella* (2 isolate), *Enterobacter*(10 isolate), *Klebsiella* (10 isolate), *Serratia* (5 isolate) and *Pseudomonad* (6 isolate).

The most effected antibiotic on all types of bacteria isolated from under the long nail was Gatifloxacin and the lowest effect was Cefazolin antibiotic.

The concept of the study was isolating and determining bacteria, found under the long fingernails and studying their antibiotic sensitivity.

Keywords: *Under Nails; Antibiotic; Bacteria Isolated ; Health.*

Introduction

The skin on the human body is in permanent contact with the environmental microorganisms, these contaminant microorganisms can easily be isolated in laboratories. These microbes can induce a range of diseases in the community or hospital, including urinary tract, respiratory tract, injuries and burns, bacteremia, neonatal meningoencephalitis, empyema and osteomyelitis. The hand acts as a significant transmission platform for different microbes, including the enteric species [1].

The most in touch human body components with the outside world are the hands. People are using their hands every day for a wide range of different activities. Contacting distinct microbes and transferring them to other objects and perhaps even individuals is highly simple. Surprisingly, the larger number of bacteria discovered on human hands are under fingernails [2]. In many health-related problems, fingernails are progressively seen as a significant concern due to the ability to harbor many kinds of microorganisms [3].

The finger nail is a significant structure consisting of the protein, keratin, laminated layers. Nails have two key roles, in spite their tiny number. They function as a protective lamina and by acting as a counter-force to improve the feeling of the fingertip. Every nail comprises several components including: nail root, nail bed, nail plate, perionychium and hyponychium [4].

Even microbes may still occur under fingernails when hands are washed. Higher microorganism populations (2 to 3 log CFU / fingernail) 10 happen commonly under the nails and are often more hard to remove than at other hand places (CDC, 2002). Length and texture of the fingernails also influence the effectiveness of microbial removal from below the nails. Long and polished nails usually contain more microbes after cleaning hands than brief and unpolished nails [5].

Therefore, using artificial fingernails can be a factor that influences the effectiveness of hand washing as artificial nails are generally longer than natural nails. Several studies have shown that greater populations of microbial are retrieved from artificial nails than natural nails [3]. Effective hand washing techniques are crucial in

stopping disease transmission via finger nails to remove microbes from artificial or natural [6].

The higher population of pathogenic microorganisms found under long nails were *Escherichia coli, Shigella, Salmonella, Enterobacter, Klebsiella, Serratia , Proteus ,Bacillus and pseudomonas* [6, 7].

Materials and Method

Collection of samples

A total of 100 samples were collected from Kindergarten students, primary, secondary and university students. This study was conducted between October 2017 and May 2018 in Al-Hillah city. The samples were gathering by sterile tooth picking, the tooth picking was scrub throughout the surface of the under-nails and moved to the brain heart infusion broth to guarantee that bacteria remained alive, after the broth was incubated at 37°c for 18 h.

Methods of isolation and diagnosis

Culturing Methods

In the research, the nutrient agar medium was used for bacterial culturing. Detailed data was gathered under the samples of the nails (lengthy nails) depending on era and sex. The crops were incubated at 37 ° C and bacterial development was inspected at 24 hours. To achieve pure culture, separate colonies were sub-cultured into nutrient agar. Shape and color of colonies (morphological characteristics) , gram stain tests and biochemical tests were used to identify bacteria.

Identification of Bacteria

Bacteria were diagnosed by using several selective media like, mannitol salt agar, maCconky agar, eosin methylene blue, SS agar and blood agar also bacteria were gram stained.

Antibiotic Susceptibility Test

Several antibiotic dicks were used (Aztreonam ATM(30mcg) ,Cefazolin CZ(30mcg), Cefotaxime CTX(30mcg), Clindamycin DA(2mcg), colistin CT(10mcg), Gatifloxacin GAT (5mcg), Nitrofuranton F(300mcg) and Trimethoprim/ Sulphamethoxazol SXT(25mcg)) to test the sensitivity of bacteria .The test was done by using Muller Hinton media.

Result and Discussion

Microorganisms existence under the nails has become the most widely health issue. A total of 100 samples from under nails by tooth picks were gathered from under the nails, 100 students were gathered in the left and right hands.

All students (100%) found that they were harboring bacteria under their nails. Bacterial pathogens that were isolated from the students ' lower nails were found in (table 1).

Table (1): Types and Number of bacteria isolated from samples under nails

No.	Gram – positive		Gram – negative	
1.	Staphylococcus sp.	56	Escherichia coli	40
2.	Bacillus sp.	1	Salmonella	2
3.	Streptococcus sp.	1	Enterobacter	10
4.			Klebsiella	10
5.			Serratia	5
6.			Pseudomonad	6
7.	Total	58	Total	73

Rayan and Flournoy Clarify the presence of large bacterial growth under fingernails over 1 mm in length, showing that volunteers with short finger nails (cut correctly) had 64% bacterial contamination (bacterial count) and volunteers with lengthy finger nails had more (67%) bacterial count presence on their hands [8].

Lin indicated that more microorganisms tend to harbor lengthy fingernails than brief nails. Visibly smooth nails were only noted by the appearance of students ' finger nails, showing 62% bacterial contamination on their hands. Ray noted there was a reduction in the bacterial isolates after washing hands with soap [9, 10].

Tambekar also found the largest bacterial contamination (70%) in the hands of Kindergarten volunteers followed by 67% in the hands of primary volunteers, 66% in the hands of secondary pupils, 64% in the hands of PG volunteers and at least (57%) in the hands of undergraduate volunteers [11].

The Microorganisms that isolated and diagnosed from fingernail tested for antibiotic sensitivity, eight antibiotic were used as mentioned in material and methods, (antibiotic resistance pattern were shown in table (2).

Table (2) antibiotic resistance pattern of isolated bacterial species from finger nail (compared with NCCLS guidelines)

No.	Bacterial strains	Clear zone diameter (mm)							
		CZ	F	CT	GAT	ATM	DA	CTX	SXT
1.	Pseudomonad	R	R	R	S	R	R	R	R
2.	Klebsiella	R	R	R	R	R	R	R	R
3.	Streptococcus sp.	R	R	R	S	R	R	S	R
4.	Escherichia coli	R	R	R	R	R	R	R	R
5.	Salmonella	R	R	S	S	S	R	R	R
6.	Staphylococcus sp.	R	S	R	S	R	S	R	R

Diagram (1) Clear zone diameter by (mm) for different strain and antibiotic types

As it is clear from the diagram above Gatifloxacin was the most effected antibiotic on the bacteria isolated from under nails and the lowest effect was belong to Cefazolin .

Kibret M. Says *E.Coli* isolates show high erythromycin, amoxicillin and tetracycline resistance levels. For experimental treatment of *E. coli*, nitrofurantoin, norflaxocin, gentamicin and ciprofloxacin are regarded suitable *E. coli* in the field of research. It is recommended to monitor regularly antimicrobial susceptibility [12] while the result of this study , *E.coli* show resistant to all antibiotics that had been studied.

The results of *Salmonella* sensitivity test show high level of resistance to cefazolin, nitrofuranton, clindamycin, cefotaxime and trimethoprim/ sulphamethoxazol and had mild sensitivity to colistin, gatifloxacin and aztreonam; Mijovic etal noticed that there were increase in the susceptibility rate to many antibiotic between their two surveys [13].

The sensitivity was tested in vitro and on the basis of laboratory results, antibiotics were given to patients. Over the past 21 years, *Klebsiella* has shown a substantial shift in sensitivity pattern. These organisms ' sensitivity to different antibiotics tested has decreased over time. The organism carries out most of the in vitro antibiotics it is subjected to. Srinivasan and his coworkers explained their efforts should be focused on reducing the use of antibiotics or a correct antibiotic policy that controls the meaningless and excessive use of antibiotics [14]. their fears about gaining bacteria multi-resistance drugs traits had been observed in this research as the *Klebsiella* isolate was resistant to all eight antibiotics used.

Pseudomonas antibiotic susceptibility test appear that *Pseudomonas* strains were resistance to almost all antibiotics and sensitive only to gatifloxacin ,as clear from this study *Pseudomonas* resistant to colistin while Sader and his coworkers maintained that *Pseudomonas* show sensitivity >90% to colistin in the time between 2012-2015 [15].

Streptococcus isolate that obtained from this study was resistant to Aztreonam ,Cefazolin, Clindamycin, colistin, Nitrofuranton and Trimethoprim/ Sulphamethoxazol , while it was sensitive to Cefotaxime , Gatifloxacin , The highest resistance *Streptococcus* showed to erythromycin, clindamycin and trimethoprim-sulfamethoxazole and these should be avoided in the treatment [16].

Staphylococcus colonies showed resistance to most antibiotic that had been used in the study as listed in table (2), *Staphylococcus* spp. Also show sensitivity to three types of antibiotics (Nitrofuranton, Gatifloxacin and Clindamycin), many researchers reported that *Staphylococcus* which isolated from finger nail have multiple antibiotic resistant [6, 17, 18].

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Willey, J.M., et al., Prescott, Harley, and Klein's Microbiology. 2008, New York: McGraw-Hill.
2. Jiaravuthisan, M.M., et al., Psoriasis of the nail: anatomy, pathology, clinical presentation, and a review of the literature on therapy. Journal of the American Academy of Dermatology, 2007. 57(1): p. 1-27.
3. Hedderwick, S.A., et al., Pathogenic Organisms Associated with Artificial Fingernails Worn by Healthcare Workers. Infect. Control Hosp. Epidemiol. Infection Control & Hospital Epidemiology, 2000. 21(8): p. 505-509.
4. Larson, E., Hygiene of the skin: when is clean too clean? Emerging infectious diseases, 2001. 7(2).
5. Gupta, A., M. Zaman, and J. Singh, Fast and sensitive detection of Trichophyton rubrum DNA from the nail samples of patients with onychomycosis by a double-round polymerase chain reaction-based assay. British Journal of Dermatology, 2007. 157(4): p. 698-703.
6. Ukaegbu-Obi, K.M., E. Enya, and C.C. Dimeke, Bacterial Profile and Antimicrobial Susceptibility Pattern of Isolates from Nails of Students of Michael Okpara University of Agriculture, Umudike. 2017.
7. Risan, M.H., Isolation and Identification of Bacteria from under Fingernails. Intl J Curr Microbiol Appl Sci, 2017. 6(8): p. 3584-3590.
8. Rayan, G. and D. Flournoy, Microbiologic flora of human fingernails. The Journal of hand surgery, 1987. 12(4): p. 605-607.
9. Lin, C., et al., Removal of Escherichia coli on hands with natural or artificial fingernails. International Journal of Epidemiology, 2002. 21: p. 1157-1164.
10. Ray, S., et al., Hand washing practices in urban and rural communities in and around Kolkata, West Bengal. Indian journal of public health, 2009. 53(3): p. 192-195.
11. Tambekar, D., Minimization of illness absenteeism in primary school students using low-cost hygiene interventions. Online Journal of Health and Allied Sciences, 2012. 11(2).
12. Kibret, M. and B. Abera, Antimicrobial susceptibility patterns of E. coli from clinical sources in northeast Ethiopia. African health sciences, 2011. 11(3): p. 40-45.
13. Mijović, G., Antibiotic susceptibility of Salmonella spp.: a comparison of two surveys with a 5 years interval. Journal of IMAB–Annual Proceeding Scientific Papers, 2012. 18(1): p. 216-219.
14. Srinivasan, S., et al., Antibiotic sensitivity pattern of Klebsiella species in burn wounds at Bai Jerbai Wadia hospital for children, Mumbai, India-a 21 year study. International journal of burns and trauma, 2017. 7(5): p. 64.
15. Sader, H.S., et al., Pseudomonas aeruginosa antimicrobial susceptibility results from four years (2012 to 2015) of the International Network for Optimal Resistance Monitoring Program in the United States. Antimicrobial agents and chemotherapy, 2017. 61(3): p. e02252-16.
16. Karcic, E., et al., Antimicrobial susceptibility/ resistance of Streptococcus pneumoniae. Materia socio-medica, 2015. 27(3): p. 180.
17. Dagnew, M., et al., Bacterial profile and antimicrobial susceptibility pattern among food handlers at Gondar University Cafeteria, Northwest Ethiopia. Journal of Infectious Diseases and Therapy, 2013. 1(2 • 1000105): p. 1-6.
18. Mengist, A., Y. Aschale, and A. Reta, Bacterial and Parasitic Assessment from Fingernails in Debre Markos, Northwest Ethiopia. Canadian Journal of Infectious Diseases and Medical Microbiology, 2018. 2018.

Removal of Tetracycline from Aqueous Solutions using Pomegranate Peels Residues Accessing to ZRL

Rajaa S. Abass[1] Suad Turky Ali[1], Tamara Sami Naji[2]

[1]Assistant Lecturer, [2]Ph.D. Dr./ Al Mamon University College, 14 Ramadan St, Baghdad, Iraq

Abstract

Antibiotics are harmful pharmaceuticals to ecosystems in general and aquatic systems in particular. Therefore, its remediation from water bodies is a topic of great importance for water treatment and purification workers. This research investigates the removal of one of the most famous types of antibiotics, which was tetracycline from simulated synthetic aqueous solutions by adsorption technique using non-toxic, low cost and available agricultural waste which was pomegranate peels. The adsorption experiments were performed in adsorption laboratory unit of batch mode at different operating conditions and laboratory temperature. The operating parameters studied included pH of solution, dose of adsorbent media, treatment time, agitation speed and initial concentration of tetracycline. The results showed the ability of pomegranate peels to extract tetracycline from aqueous solutions with high efficiency of 81.55%. The results also showed that the percentage of antibiotic adsorption from aqueous solutions was inversely correlated with increasing the initial concentration and acidic function of the tetracycline solution while it was directly proportional to the amount of pomegranate peels, agitation speed and treatment time. In this style, one of the most important types of antibiotics that contaminated water was disposed of by a cheap material and using a simple, economical and environmentally friendly method accessing to the principle of zero residue level (ZRL).

Keywords: *Antibiotic, Tetracycline, Adsorption, Pomegranate peels, ZRL*

Introduction

Antibiotics are defined as chemical compounds used in the treatment, prevention and diagnosis of diseases, thus preserving the physical and mental health of both humans and animals [(1)]. Today, these products pose a threat to humans and the environment as they are increasingly present in the aquatic environment, even at low concentrations of up to parts per million (ppm) as a result of increased production due to overconsumption without any treatment before disposing [(2)]. It enters wastewater with urine and excrement as well as industrial waste from pharmaceutical plants also; their environmental impact is increasing when there is a mixture of these substances with metabolites [(3)]. Recent studies have confirmed the inclusion of these formulations as contaminants, as they pose a threat to groundwater and surface water, thus causing adverse effects on wildlife and aquatic life [(4)]. These compounds include, for example, Nonsteroidal anti-inflammatory drugs (NSAIDs), antipyretics, antidepressants, diuretics, antibiotics, and anti-ulcers, whose metabolism produces new compounds that are also polluting the environment. Antibiotics come out with urine in low concentrations, and their metabolites come out with either with urine or excrement [(5)]. Conventional methods of wastewater treatment are ineffective in eliminating the contaminated effect of pharmaceutical compounds due to the resistance of some types or metabolic products to biodegradation [(6)]. Apart from traditional treatment methods, adsorption technology has recently received widespread attention as one of the candidate methods for solving the problem of water contaminated by antibiotics for its ease, efficiency and low cost [(7)]. Studies have shown that the use of adsorption technique by activated carbon is very effective in the disposal of many organic pollutants such as dyes, pesticides and aromatic compounds generally [(8)]. However, there are two problems facing this promising technique: the first problem is the high production cost of activated carbon and needed for continuous regeneration process, in addition to the part loss of this material during each regeneration process, while the second problem relates to the difficulty of sediments

disposal from the surface of activated carbon or other adsorbents [9]. This led the researchers to seek for other sources to be used as adsorbents or raw materials in the preparation of activated carbon from them and also so that the amount of remaining materials are small [10]. In the last years, the concept of zero residue level (ZRL) has been applied to remedy all the problems associated with the use of adsorption technology. This concept uses the non-valuable waste as adsorption media and then utilizes from the residue adsorption process so that the amount of residual waste is close to zero [11]. The present paper aims to use the adsorption technique and the application of the principle of ZRL in the treatment of water contaminated with tetracycline, one of the common types of antibiotics through the use of pomegranate peels as a low-cost adsorbent in an economical, beneficial, low-cost and environmentally friendly method.

2. Experimental Work

Materials

2.1.1 Pomegranate peels (sorption media): The mature pomegranate peels used in this investigation were obtained from juice shops and cafes in Baghdad city as well as from domestic usage. After collection, pomegranate peels (in its originally size) were washed with excess tap water for several times before being washed with distilled water at normal temperature to get rid of any kind of impurities and dust that might be stuck to them. The washed peels were dried naturally by exposing them to the open air and sunlight for uninterrupted 48 hours and then placed in a metal bowl, immersed in fresh water and heated until boiled to remove the color and dye (tanner) from them. Finally kept in dark brown glass bottles and placed in the fridge until used.

3.1.2 Stock solutions: Real water contaminated with tetracycline (a studied antibiotic) contains many compounds and other elements that may be difficult to detect exactly or identify accurately in that type of water. Therefore, the adsorption experiments were carried out using simulated synthetic aqueous solutions (SSAS) containing tetracycline in various concentrations in order to evade nip up with any other kind of pollutants. For this purpose, an aqueous solution, called stock solution, of 1000 ppm of tetracycline, was prepared. In a volume of one liter of distilled water, 1 g of tetracycline powder packaged in capsules was dissolved to obtain the stock solution. All different concentrations SSAS of tetracycline antibiotic used in this research were prepared from dilution of the stock solution with distilled water to the desired concentration. Tetracycline concentrations were determined spectrometrically at a wavelength of λ=529 nm using a spectrophotometer according to the method described by [12]**.** Figure 1 shows the spectrophotometer calibration curve intended for tetracycline concentrations.

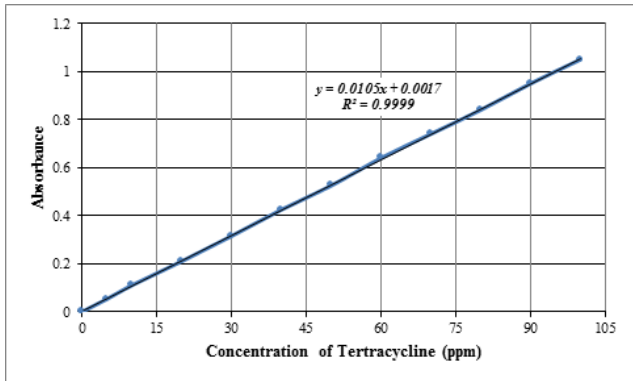


Figure 1 Spectrophotometer calibration curve of tetracycline @ wavelength of λ=529 nm

3.2 Adsorption unit: In order to identify the behavior of pomegranate peels as an adsorption media and to determine the best operational conditions that achieve the maximum tetracycline removal percentage, a concatenation of functional experiments were conducted in a laboratory adsorption unit of batch mode. In each experiment 100 ml of tetracycline solution was prepared and laboratory experiments were carried out at different operational conditions of initial concentration of tetracycline, pH of SSAS, amount of adsorbent, agitation speed and contact time. Their ranges were from (1-50) ppm and (1-8) (0.25-2.5) g of pomegranate peels, (100-400) rpm and (10-150) min. respectively and at laboratory temperature (28±2) °C. Each experiment was triplicate to increase accuracy and to reduce the experimental error. To calculate the residual tetracycline concentration at the end of the experiment, the aqueous solution was filtered using vacuum filtration to separate any residue of pomegranate peels may be present in the treated solution. A sample of the filtered aqueous solution was drawn, tested by the spectrophotometer and the concentration of the antibiotic removed was detected. The efficiency of tetracycline removal from the SSAS was determined by calculating the percentage of removal that can be found from the following mathematical relationship:

$$\%R = \frac{C_i - C_f}{C_i} \times 100$$

Where:: refers to tetracycline percentage removal, and : refer to initial and final concentration of tetracycline (ppm) respectively.

Results and Discussions

As illustrated above, the study of adsorption technology as a suggested remediation method for SSAS contaminated with tetracycline antibiotic was conducted in a batch adsorption unit. The removal process was examined at different operating conditions and using pomegranate peels as a cheap and available adsorbent. This section discusses the effect of the operational conditions used on the tetracycline removal efficiency and determination of the optimum conditions for the maximum treatment efficiency of contaminated SSAS.

3.1 Effect of Initial Concentration of Tetracycline: The results obtained from the experiments of changes the initial concentration to remove tetracycline using pomegranate peels as an adsorption media from SSAS showed that the percentage removal was increased by decreasing the value of the initial concentration and vice versa. The results also showed that the maximum percentage removal was 81.46% at the lowest initial concentration of 1 ppm while the percentage removal at the highest studied concentration which was 50 ppm was 23.19% as explained in Figure 2. Adsorption technology is great dependent on surface area and it is a constant property of adsorption media, representing the available sites on the surface of the adsorbent material at which adsorption process occurs. The number of these active sites is limited in the adsorbent and has a constant adsorption capacity for a specified number of contaminated matter particles. In the case of low concentrations of tetracycline, the pomegranate peels was able to adsorb more molecules than if the antibiotic concentration is higher; i.e., the number of non-adsorbed tetracycline molecules that will remain free in the solution will be lower at low concentrations at constant volume and therefore the efficiency of pomegranate peels as a medium of adsorption to remove tetracycline will be reduced by increasing the initial concentration of the contaminant in aqueous solutions.

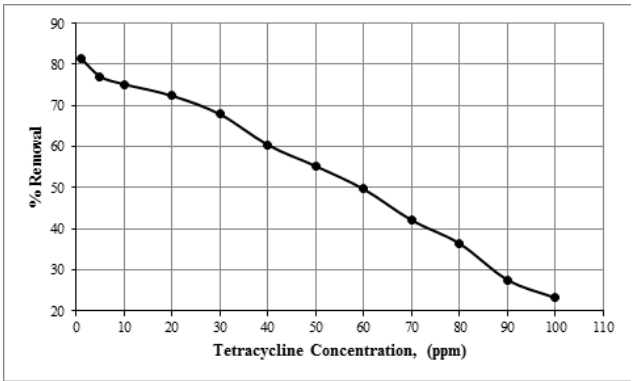


Figure 2 Effect of initial concentration on the percentage removal of tetracycline antibiotic

3.2 Effect of pH: Figure 3 shows the behavior of tetracycline adsorption process by pomegranate peels when the pH of SSAS is changed. It's obvious from above Figure that the relationship between the percentage removal and the pH of solution is inversely and the maximum percentage removal was obtained at the lowest pH value. The pH has a clear effect on the adsorption process, as it affects the charge and ionization degree of the active sites at the surface of adsorbent media. This may be due to the dependence of tetracycline ionization on the value of the pH. On the other hand, increasing the pH leads to an increase in the concentration of negative hydroxide ions (OH^-) in the solution, which generates repulsive forces between them and tetracycline molecules and thus competes for the active sites in the adsorbent, which is already limited. Therefore, the high percentage removal means that there are little competition and repulsion forces between tetracycline and hydroxide. In addition, the surface of the pomegranate peels will be ionized with positive hydrogen ions. This makes the adsorption process easier than if the number of hydroxide ions increases. This happens when the acidic function of the solution is raised.

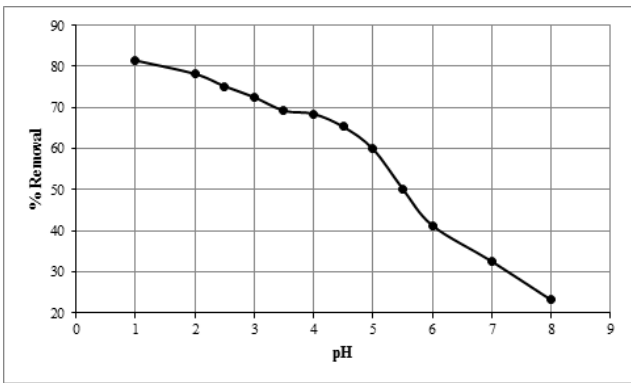


Figure 3 Effect of pH on the percentage removal of tetracycline antibiotic

3.3 Effect of Adsorbent Amount: The experimental results related to the study of the pomegranate peels effect as an adsorbent on the percentage of adsorption showed that the latter is increasing by increasing the amount of adsorbent by keeping the other operational parameters at optimum values. The maximum removal was recorded at the largest amount of adsorbent, which is 2.5 g, as shown in Figure 4. The direct correlation between the percentage of tetracycline removal and the amount of pomegranate peels is due to the fact that pomegranate peels have a specific surface area per unit weight. Increasing the amount of adsorbent will increase the surface area of the adsorption medium, which means more active sites, which in turn will provide a greater chance of adsorption of more molecules of tetracycline if the amount of pomegranate peels is less.

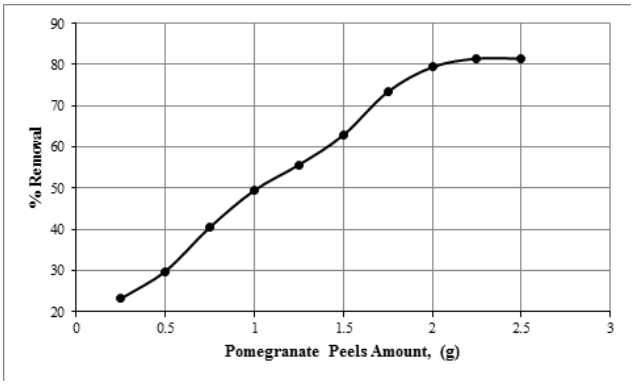


Figure 4 Effect of adsorbent media amount on the percentage removal of tetracycline antibiotic

3.4 Effect of Agitation Speed: The correlation between the percentage removal of tetracycline and different values of agitation speed is shown by Figure 5. Increasing the agitation speed will increase tetracycline removal for from aqueous solution using pomegranate peels as adsorption medium and keep the rest of the operational variables at optimal values. This results may be attributed for Increasing the agitation speed will reduce the thickness of the layer surrounding the adsorbent molecules, removing the surrounding obstacles and increasing the chance that tetracycline will bind to the active sites on the surface of the pomegranate peels, thus increasing the tetracycline removal from the aqueous solutions. This explanation is true up to the speed of 300 rpm, after this value the percentage removal remains constant and does never change whatever increasing the agitation speed. This may be due to the material is saturated with adsorbed molecules at optimum speed and that any increase in speed will not change the removal efficiency.

3.5 Effect of Contact Time: The increase in contact time leads to a corresponding increase in the percentage of tetracycline removal from aqueous solutions as shown in Figure 6, with keeping the rest of the operational variables at optimal values. Increasing the process time will increase the time when tetracycline molecules come into contact with pomegranate peels, which in turn will increase the chance of tetracycline binding to active sites on the surface of the adsorbent and thus increase the rate of antibiotic removal from aqueous solutions. If the time is less, vice versa, the adsorbent molecules do not find the time required to complete the adsorption process of the molecules of the contaminant and thus remain free in the solution, which leads to a decrease in the percentage of adsorption.

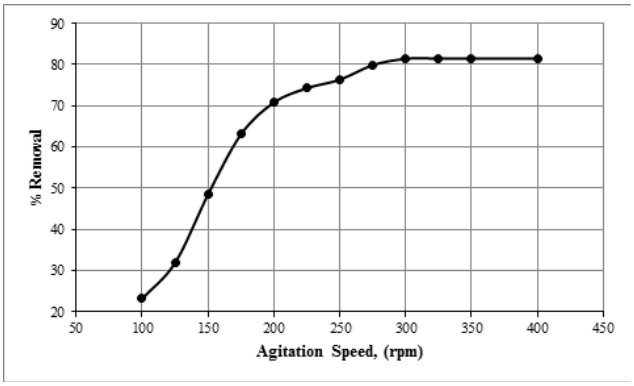


Figure 5 Effect of agitation speed on the percentage removal of tetracycline

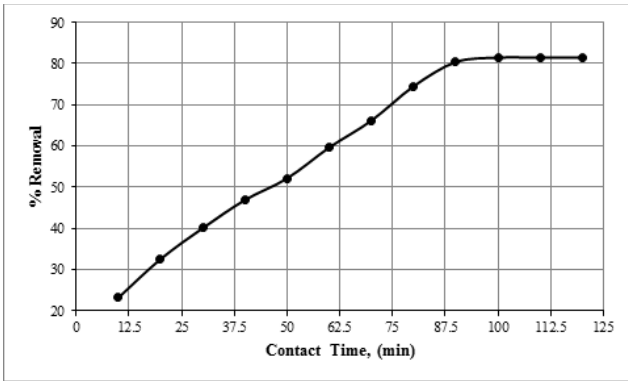


Figure 6 Effect of contact time on the percentage removal of tetracycline

4. Conclusions: From the results of the present study, the following conclusions can be drawn:

a. Adsorption technique showed high efficiency in the treatment of aqueous solutions contaminated with antibiotics in general and tetracycline in particular.

b. The maximum percentage removal was 81.46% at 1 ppm of the initial concentration of tetracycline, pH of 1, pomegranate peels amounts of 2.5 g, agitation

speed of 300 rpm and contact time of 100 min.

c. Adsorption of tetracycline from aqueous solutions using pomegranate peels was directly proportional with the amount of adsorbent (pomegranate peels), agitation speed and contact time to a certain level and then constant. While the percentage removal was inversely proportional to the initial concentration of tetracycline and the pH of the aqueous solution.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

(1) Gallagher JC and MacDougall C. Antibiotics Simplified-Jones & Bartlett Learning. 2012. 2nd Edition, Jones & Bartlett Learning. ISBN: 978-1-4496-1459-1

(2) Leng L, Wei L, Xiong Q, Xu S, Li W, Lv S, Lu Q, Wan L, Wen Z and Zhou W. Use of microalgae based technology for the removal of antibiotics from wastewater: A review. Chemosphere. 2020;238, Article 124680. https://doi.org/10.1016/j.chemosphere.2019.124680

(3) Kumari M and Kumar A. Human health risk assessment of antibiotics in binary mixtures for finished drinking water. Chemosphere. 2020;240, Article 124864. https://doi.org/10.1016/j.chemosphere.2019.124864

(4) Bengtsson-Palme J, Milakovic M, Švecová H, Ganjto M, Jonsson V, Grabic R and Udikovic-Kolic N. Industrial wastewater treatment plant enriches antibiotic resistance genes and alters the structure of microbial communities. Water Research. 2019;162:437-445. https://doi.org/10.1016/j.watres.2019.06.073

(5) Patel M, Kumar R, Kishor K, Mlsna T, Pittman CU and Mohan D. Pharmaceuticals of Emerging Concern in Aquatic Systems: Chemistry, Occurrence, Effects, and Removal Methods. Chemical Reviews. 2019;119(6):3510-3673. https://doi.org/10.1021/acs.chemrev.8b00299

(6) Kumar M, Jaiswal S, Sodhi KK, Shree P, Kumar D, Kumar SP and Shukla AP. Antibiotics bioremediation: Perspectives on its ecotoxicity and resistance. Environment International, 2019;124:448-461. https://doi.org/10.1016/j.envint.2018.12.065

(7) Zhang M, Xu L, Qi C and Zhang M. High effective removal of tetracycline from water by hierarchical porous carbon: Batch and column adsorption. Industrial & Engineering Chemistry Research, 2019; Just Accepted Publication Date (Web): October 1. https://doi.org/10.1021/acs.iecr.9b03547

(8) Fraga TJM, Carvalho MN, Ghislandi MG and Sobrinho MAd-M. Functionalized Graphene-Based Materials as Innovative Adsorbents of Organic Pollutants: A Concise Overview. Brazilian Journal of Chemical Engineering 2019;36(01):1-31. http://dx.doi.org/10.1590/0104-6632.20190361s20180283

(9) Lach J. Adsorption of Chloramphenicol on Commercial and Modified Activated Carbons. Water. 2019;11(6):1141, May. http://doi.org/10.3390/w11061141

(10) Saleem J, Bin Shahid U, Hijab M, Mackey H and McKay G. Production and applications of activated carbons as adsorbents from olive stones. Biomass Conversion and Biorefinery. https://doi.org/10.1007/s13399-019-00473-7

(11) Alalwan HA, Abbas MN, and Alminshid AH. Uptake of Cyanide Compounds from Aqueous Solutions by Lemon Peel with Utilising the Residue Absorbents as Rodenticide. Indian Chemical Engineer. 2019 https://doi.org/10.1080/00194506.2019.1623091

(12) Hadi H and Fadhil G. Sensitive Spectrophotometric Determination of Tetracycline Hydrochloride Indosage forms using Sodium Nitroprusside and Hydroxylamine Hydrochloride. Journal of Al-Nahrain University/Science. 2014;17(3):53-58

Complications of Laparoscopic Cholecystectomy in a Sample of Patients Admitted to Al-Ramadi Teaching Hospital, Anbar-Iraq

Tarek Mahdi Salih

Assist prof. F.I.C.M.S., D.G.S., College of Medicine, University of Anbar /Iraq

Abstract

Laparoscopic cholecystectomy is currently thought as a surgical procedure that can be done with less risk of complications. However, intraoperative and postoperative complications were existing in 56 (15.01%) patients. Open surgery is the case when laparoscopic cholecystectomy fails to be proceeded with due to many causes such as difficult Calot triangle dissection and empyema of gallbladder.

The high occurrence of cholecystitis in the group of patients aged 40-60 (56%) with 151 female and 59 males initiating the floor to many arguments that may help give a good understanding for such a problem.

Iatrogenic perforation of gallbladder was the most common cause of intraoperative complications. Wound infection was the most cause of postoperative complications. Difficult Calot triangle dissection was the most common cause to convert to open surgery.

Keywords: *Hospital; Laparoscopic cholecystectomy ; Infection.*

Introduction

In the treatment of benign gallbladder disease, laparoscopic cholecystectomy became the first choice that may be taken instead of open surgery[1,2]. On the other hand, surgeons believed that this procedure involved high risk of injury compared to open cholecystectomy[3,4]. In this context, experienced surgeons are needed to proceed with such new technique of treatment.

It has been estimated that in USA about one million patients annually diagnosed with gallbladder disease in which about 75% of them underwent laparoscopic cholecystectomy[5].

Complications of this treatment procedure may vary according to the health status of patients, experience of surgeons, the post-operative care, as well as many other things that may affect negatively the success of the procedure. In this study a light will be casted on most of the common complications as recorded from patients underwent laparoscopic cholecystectomy.

In this technique a gallbladder is removed by a key-hole-sized incision usually using two 10mm ports and two 5mm ports in which this procedure believed to result with less postoperative complications[6].

This study aims to evaluate the intraoperative, post -operative complications and rate of conversion treatment in patients with cholecystitis.

Patients and Method

During the period Jan. 2012 to the end of Dec. 2013, 373 patients (102 male and 271 female) with history of cholecystitis admitted to AL-Ramadi General Teaching Hospital, Anbar province, Iraq, were considered in this study.

Data collected from each patient included age, gender, clinical signs and symptoms as well as relevant examinations. Medical investigations such as WBC counts, ultrasound findings, postoperative histopathological findings of gallbladder were recorded.

The collected data were classified in tables according to the purpose of presentation and descriptive and inferential statistics were made whenever needed in this paper.

Results

Table 1 shows the distribution of patients according to age groups with reference to their gender groups. Age ranged between 18-70 years with mean age of 49.28 years. Most of the patients were female and accounted for 72.65%. Patients aged 40-60 constitute the highest percentage among all other.

Table 1. Distribution of patients according to gender and age groups.

Age in years	Male	Female	Total	%
Less than 20	0	6	6	2
20-40	31	82	113	30
40-60	59	151	210	56
More than 60	12	32	44	12
Total	102	271	373	100

Out of the total patients involved in this study, 56 (15.01%) showed different complications. Table 2 shows the distribution of those patients according to gender and age groups. Males were accounted for relatively higher percentage 55.4% compared to 44.6% of females. About 88% of those patients were in the ages of 40 years or more.

Table 2. Distribution of patients with complications according to age and gender groups.

Age in years	Male	Female	Total	%
Less than 20	0	0	0	0
20-40	4	3	7	12.5
40-60	19	9	28	50.0
More than 60	8	13	21	37.5
Total	31	25	56	100.0

Recorded complications are classified into two main categories, intraoperative and postoperative as presented in table 3. Out of the total patients of this study, 22 (5.9%) revealed intraoperative complications whereas 34 (9.12%) showed postoperative complications. Out of the intraoperative complications, 7 cases (22.58%) found to have vascular injuries. Iatrogenic perforation of GB was accounted for the highest percentage (41.94%) among all other types of intraoperative complications.

With regard to postoperative complications, wound infection was found the most common complication which accounted for 30.43% followed by bleeding from abdominal cavity with 21.74%.

Table 3. Intraoperative and postoperative complications classified by gender groups (n=373).

Complications		Males	Females	Total	%
Intraoperative:					
Vascular injury	bleeding from bed of GB	3	2	5	1.34
	bleeding from cystic artery	1	0	1	0.27
	bleeding from port site	1	0	1	0.27

Cont... **Table 3. Intraoperative and postoperative complications classified by gender groups (n=373).**

Iatrogenic perforation of GB	6	7	13	3.49
Spillage of gall stones	3	4	7	1.88
Bowel injury	1	1	2	0.54
Common bile duct injury	1	0	1	0.27
Transection of common hepatic duct	1	0	1	0.27
Total	17	14	31	8.31
Postoperative:				
Bleeding from abdominal cavity	7	3	10	2.68
Bile leak	4	3	7	1.88
Retained stone in CBD	0	1	1	0.27
Port hernia	1	1	2	0.54
Wound infection	8	6	14	3.75
Lost gall stone in abdominal cavity	3	1	4	1.07
Pneumonia	3	2	5	1.34
Pulmonary embolism	1	0	1	0.27
Deep vein thrombosis	1	1	2	0.54
Total	28	18	46	12.33

Causes of conversion to open surgery are not identical, rather they vary from patient to another. In this study, only 20 (5.36%) patients were transferred to open surgery under different reasons as presented in table 4. The most common cause was difficult calot triangle dissection which accounted for 35% followed empyema of gall bladder with 25%.

Table 4. Causes of conversion to open surgery (n=373).

Causes of conversion to open surgery	Males	Females	Total	%
Difficult calot triangle dissection	4	3	7	1.88
Empyema of gall bladder	2	3	5	1.34
Bleeding from vascular supply	2	1	3	0.8
Mirrizzi syndrome	1		1	0.27
Injury to CBD	1		1	0.27
Injury to common hepatic duct		1	1	0.27
Cholecystoduedenal fistula	1		1	0.27
Colonic injuries		1	1	0.27
Total	11	9	20	5.36

It may be worthwhile referring the cases that presented with different complications to the medical procedure on which the decision of laparoscopic cholecystectomy was decided. In this context, table 5 showed the cases according to the type of complication.

With regard to WBC counts, cases with more than 10000/mm^3 were more than others for all categories of complications. Acute cholecystitis was accounted for greater number of patients at all categories of complications for both ultrasound and histopathological findings.

Table 5. Cases with different complications classified with respect to medical procedures (n=373).

Variables	Complications		Conversion to open surgery
	Intraoperative	Postoperative	
WBC count			
More than 10000/mm3	15 (4.02%)	22 (5.9%)	12 (3.22%)
Less than 10000/mm3	7 (1.9%)	12 (3.22%)	8 (2.14%)
Ultrasound findings			
Chronic cholecystitis	9 (2.41%)	13 (3.5%)	6 (1.61%)
Acute cholecystitis empyema	13 (3.5%)	21 (5.63%)	14 (3.75%)
Histopathological findings			
Chronic cholecystitis	10 (2.68%)	15 (4.02%)	9 (2.41%)
Acute cholecystitis	12 (3.22%)	19 (5.09%)	11 (2.95%)

Discussion

Since its first introduction in 1985, laparoscopic cholecystectomy is rapidly becoming very common technique. This technique enables surgeons to avoid complications of open surgery as well as the high cost of staying at hospitals waiting for recovery.

Most of the patients considered in this study were in the range of 18-70 years with mean age of 49.28 years. This mean age is less than that obtained by Chay CH, et al., 2006 (56.9 years) which also showed a wider age range (23-89 years)[7]. However, mean age of patients considered in this study is found to be less than that obtained by Al-Salamah SM, 2005[8]. Such a discrepancy can be either attributed to the sampling technique used to select the right patient, or to a certain situation that maybe the sample' population concerned about. In Iraqi communities, people after 20 years of age become responsible about their families affairs and that they have to do more efforts in order to put their families in a good economic stands. As a result, they become gradually confronted with different health problems due to the food which is not healthy prepared or even do not suit their real needs.

Vascular injury is one of the intraoperative complications, the percentage of this type of complication in this study was found to be 1.88 which is much lower than that (9.97) obtained by Rooh-ul-Muqim, et al,2008[9].

Iatrogenic perforation of gallbladder was found to have the highest percentage (3.49) among all other causes on intraoperative complications. This percentage is doubtless significantly lower (25.5) than that obtained by Zubair M, et al, 2010[10]. Iatrogenic perforation of gallbladder may be associated with the adhesion in right upper quadrant of gallbladder, or with other variables such as gender which is not the aim of this study.

Spillage of gall stones was found in 1.88% of patients in this study. This percentage was also much lower than that obtained by Zubair M, et al, 2010 which was 11.5. However, the percentage 1.88 was almost similar to that (2.02) obtained by Miodrag Radunovic, et al, 2016[11].

It is believed that the association of iatrogenic perforation of gallbladder and spillage of gall stones may

lead to abdominal infections which in turn can result in a number of abdominal problems[12].

With regard to the postoperative complications, the most type was found to be wound infection which was found in 14 (3.75%) patients of this study. Such problem may be caused by poor hygiene or to polluted environment where the patient(s) moved to after surgery. The second cause of postoperative complication was bleeding from abdominal cavity which occur in 10 (2.68%) patients in this study. The third type of postoperative complications in this study was the bile leak which found in 7 (1.88%) patients which is less occur when compared to 3.98% of patients in the study conducted by Rooh-ul-Muqim, et al,2008[9].

Other types of postoperative types as found by this study seems very rare and the deep vein thrombosis do not appear to be mentioned in literature reviewed during the research.

Causes of conversion to open surgery are also vary according to the patient situation as well as potential complications during key-hole-surgery. However, in this study the main causes were difficult calot triangle dissection which was seen in 1.88% of the patients and empyema of gallbladder which seen in 1.34% of the patients. In general, 5.36% of the patients of this study were converted to open surgery and when compared to 3.13% that found by Rooh-ul-Muqim, et al,2008 [9] , one may easily conclude that patients of this study were actually confronted by many problems that make their health stands in a serious situation.

Number of patients with intraoperative and postoperative complications as well as of those converted to open surgery, were cross-classified with respect to medical signs and findings. Patients with WBC counts more than 10000/mm^3 are remarkable more than those with WBC counts lower than 10000/mm^3. Acute cholecystitis was also found in a number of cases that essentially exceeds those with chronic cholecystitis. This is true for both ultrasound and histopathological findings.

Conclusion

Laparoscopic cholecystectomy is indeed well prevailed in surgical practice. It is not always the only good procedure to follow but a procedure with less pain and potential complications. Nevertheless, over the wide use of this procedure, different types of complications were recorded. Actually, complications may appear due to variety of factors including the health status of the patients.

In this study most of the admitted cases to undergo laparoscopic cholecystectomy were over 29 years of age and 68% of them were above 40 years old with male to female ration of 0.38:1.

Complications found in 56 of the patients, of them 31 males and 25 females.

Iatrogenic perforation of gallbladder, spillage of gall stones and vascular injury were the most common causes of intraoperative complications. On the other hand, wound infection, bleeding from abdominal cavity, bile leak, pneumonia and loss of gall stones in abdominal cavity were the most common causes of postoperative complications.

Difficult calot triangle dissection and empyema of gallbladder were the most common causes of conversion to open surgery.

Most of those converted to open surgery have WBC counts more than 10000/mm^3 and acute cholecystitis in both ultrasound and histopathological findings.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Ros A, Carlsson P, Rahmqvist M, Bachman K, Nilsson E. Nonrandomized patients in a cholecystectomy trial: characteristics, procedure, and outcomes. BMC Surge 2006; 6:17.

2. Ji W, Li LT, Li JS. Role of Laparoscopic subtotal cholecystectomy in the treatment of complicated cholecystitis. Hepatobiliary Pancreat Dis Int 2006;5(4):584-9.

3. Hobbs MS, Mai Q, Knuimam MW, et al. Surgeon experience and trends in intraoperative complications in laparoscopic cholecystectomy. BJS 2006;93;844-53.

4. Hasl DM, Ruiz OR, Baumert J, Gerace C, et al. A prospective study of bile leaks after laparoscopic cholecystectomy. SurgEndosc 2001;15:1299-1300.
5. Shamiyeh A, Wanyand W. Laparoscopic cholecystectomy: early and late complication and their treatment, Langenbecks arch. Surg 2004;389:164-17.
6. Gurusamy K, Vaughan J, Rossi M, Davidson B (2014) Fewer-than-four ports versus four ports for laparoscopic cholecystectomy. Cochrane Database Syst Rev (2): CD007109.
7. Chau CH, Siu WT, Tang CN, Ha PY, et al. Laparoscopic cholecystectomy for acute cholecystitis: the evolving trend in an institution. Asian J Surg 2006;29(3):120-4.
8. Al-Salamah SM. Outcome of laparoscopic cholecystectomy in acute cholecystitis. J Coll Physicians Surg Pak 2005;15(7):400-3.
9. Rooh-ul-Muqim, Qutab-e-Alam Jan, Mohammad Zarin, Mehmud Aurangzaib, et al. Complications of laparoscopic cholecystectomy. World Journal of Laparoscopic Surgery, January-April 2008; 1(1):1-5.
10. Zubair M, Habib L, Mirza MR, Channa MA, et al. Iatrogenic Gall Bladder Perforations in Laparoscopic Cholecystectomy: An Audit of 200 cases. Mymensingh Med J 2010 Jul; 19(3).
11. Miodrag Radunovic, Ranko Lazovic, Natasa Popovic, Milorad Magdelinic, et al. Complications of Laparoscopic Cholecystectomy: Our Experience from a Retrospective Analysis. Open Access Maced J Med Sci 2016 Dec 15; 4(4):641-646.
12. Zubair M, Habib L, Mirza M. Abdominal wall complications of missed gall stones in laparoscopic cholecystectomy. JDUHS. 2008;2(2):85-3.

Physiological Study Comprising the Sequelae of Magnetic Radiation on Human

Wissam Sajid Hashim[1], Azal Hamoody Jumaa[2], Noor Thamer Alsaadi [1], Ayad Gaber Arean[1]

[1]College of Medicine,– Al Muthanna University / Iraq, [2] Al-Yarmouk University College/Ministry of Higher Education and scientific Research / Iraq

Abstract

The sequelae of magnetic radiation of the towers of cellular phones were evaluated in this study depending upon a random social volunteer human male samples of ages 25 – 50 years and they were dwelling in houses close to the towers of cellular phones of distances not more than 150 miters far. The samples of these people were labelled as "Subjected". Seventy five samples of blood were gained on a base of ages as twenty five from ages 25 – 30, twenty five from ages 35 – 40, and twenty five from ages 45 – 50 in addition to seventy five samples of people who did not dwell close to the towers and of the same previous pattern of ages and their samples were labelled as "Non subjected". The results have demonstrated a significant elevation in transaminases (AST , ALT), blood calcium (Ca^{++}), blood potassium (K^{+}), total serum cholesterol (TSCH), triacylglycerols (TAGs), very low density lipoprotein (VLDL), and low density lipoprotein (LDL) .

Keywords: *Magnetic waves, Radiation, Lipids.*

Introduction

In our recent life there is an increasing need for the use of cellular phones without the ability to get rid of them taking into our mind that these phones operate on base of radiofrequency emitting and receiving ranging from 400 to 2000 megahertz [(1)]. We know that the radiofrequency usage is not limited to the phones but it is also comprised in the medical appliances and therapy use [(2)]. The stress effects on different body systems is well known and the same like occur when the body is subjected to radiofrequency due to impact energy absorption which could afflict the cellular membranes structures and cause sever damages to various cellular and subcellular structures and body organs like the effects on glands such as pituitary, adrenal, hypothalamus and others and you know this will afflict the functions of the body as a whole [(3)]. Lipid profile, liver antioxidant enzymes, hepatic enzymes, renal functions, neuronal milieu, and others all are subjected to the risk of radiofrequency [(4, 5, 6)].

Correspondent author:
Wissam Sajid Hashim
Email: dr.wissam2013@gmail.com ;
dr.w80@mu.edu.iq

Materials and Method

Specimens' collection

The specimens of blood were gained randomly from social volunteer human males of ages 25 – 50 years who were dwelling in Baghdad governorate close to the towers of cellular phones of distances not more than 150 miters far. The samples of these people were labelled as "Subjected". Seventy five samples of blood were gained on a base of ages as twenty five from ages 25 – 30, twenty five from ages 35 – 40, and twenty five from ages 45 – 50 in addition to seventy five samples of people who did not dwell close to the towers and of the same previous pattern of ages and their samples were labelled as "Non subjected". Once blood samples were collected as 5 ml/ people by the use of disposable syringes, they were poured into gel tubes to get blood serum to accomplish the necessary study parameters.

Study parameters

Special kits and a UV spectrophotometer (Apel – PD 303 UV, Japan) were used to perform all the study parameters.

► Total Serum Cholesterol, TCH (mg/dl)

Total serum cholesterol (TSCH) was estimated by using a special kit (Spinreact/CHOD – POD, SPAIN) according to the method of [7].

► Low density lipoprotein, LDL (mg/dl)

Serum LDL was obtained according to the formula: LDL-C = TC – HDL-C – TAG/5 [8].

► High density lipoprotein, HDL (mg/dl)

This was measured by using a chemical kit (HDL-Cholesterol (PTA) / Biolabo SA, France) according to [9].

► Serum very low density lipoprotein-cholesterol, VLDL (mg/dl).

Serum very low density lipoprotein was calculated by method of [8].VLDL = TAG / 5VLDL (mg/dl).

► Triacylglycerols (TAGs)

Triglycerides (TGs) are estimated by using a chemical kit (Triglycerides (GPO) / BIOLABO SA, France), depending upon the method of [10, 11].

► Blood Calcium level (mg/dl)

Blood Calcium level was estimated by the use of a special kit (Biomaghreb) according to method of [12].

► Blood Potassium level (mEq / l)

Blood Potassium level was estimated by the use of a special kit (Cypress Diagnostics) based on the method mentioned by [10, 13].

► Serum transaminases activity determination (Unit/ml)

ALT and AST enzymes were determined by the use of a special kit (Biomerrioux, Lyon-France) according to method of [14].

► Statistical analysis

Anova tests was depended to find the least significant differences (LSD) among groups by the use of SPSS version 21 program. *Numbers in tables represent the mean ± standard deviation.*

Results and Discussion

It is obvious when looking at the results (tables, 1 and 2) that all the human ages groups when continuously subjected to the radiofrequency of cellular phone towers have shown a clear significant elevations in serum AST, ALT, TSCH, LDL, VLDL, K^+, and Ca^{++} besides a significant declination in serum HDL comparing them with the values of people who were not continuously subjected to the radiofrequency of towers at ($P \leq 0.05$).

Dyslipidemia might be caused by obesity or due to consuming medications [15] and it was found that any disturbance in lipid metabolism could result in vascular pathological diseases [16]. Exposure to magnetic waves could result in different stages of peroxidation of lipids and formation of reactive radicals like oxygen reactive radicals and nitrogen ones and both of them besides the peroxidation could affect the different cellular compartments and affect also the lipid metabolism and carrying vehicles [17, 23]. The hepatic enzymes AST and ALT are considered as markers of stress and destruction of different body tissues since they are formed also by another boy regions or tissues like muscles, kidney, and heart [18, 19]. It was found that the exposure to radiofrequencies could result in elevations in cortisol which is a predisposing of stress besides their effects on redox cycles and peroxides production and hence the radiofrequencies cause the elevations of AST and ALT [22]. The elevations of blood calcium and potassium could result also by the frequency exposure as that these ions of great equilibrium in intracellular and extracellular compartments and they are carrying positive charges making the attracted to the negatively charged cellular membranes hence the disturbances caused by radiofrequency to the cell membranes and the destruction of them making the affinity towards these ions to be declined and much more ions could escape to extracellular compartments making them elevated in serum [20, 21].

Table (1). Human hepatic enzymes and electrolytes are affected by radiofrequency of mobile towers

Ages (Years)	Groups	ALT (Unit/ml)	AST (Unit/ml)	Ca++ (mg/dl)	K+ (mEq / l)
20 - 25	Non subjected	b 46.4 ± 10.8	b 39 ± 12.8	b 9.5 ± 2.8	b 4.7 ± 2
	Subjected	a 63.6 ± 15.1	a 75.1 ± 10.7	a 109.4 ± 20.8	a 9.8 ± 2
30 - 35	Non subjected	b 44.5 ± 5.4	b 31.1 ± 6.4	b 10.7 ± 3	b 4.7 ± 2.1
	Subjected	a 63.8 ± 8.5	a 78.5 ± 8.6	a 107.3 ± 20.4	a 8.2 ± 3.6
40 - 45	Non subjected	b 44.5 ± 5.4	b 32.1 ± 7.4	b 11 ± 2.7	b 4.5 ± 3
	Subjected	a 69.5 ± 12.4	a 80.7 ± 12.5	a 109.5 ± 22.7	a 8 ± 3.9
LSD		10.7	35.1	97.3	3.16

Table (2). Human lipid profile is affected by radiofrequency of mobile towers

Ages (Years)	Groups	TCH (mg/dl)	TAGs (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
20 – 25	Non subjected	d 147.5 ± 8.7	d 127.5 ± 8.9	c 97.5 ± 8.9	c 24.1 ± 1.8	d 25.7 ± 1.7
	Subjected	b 340.2 ± 22.5	b 225.2 ± 21.7	b 163.5 ± 18	a 137.5 ± 3.3	b 45.2 ± 4.5
30 – 35	Non subjected	d 168.5 ± 17	d 138.7 ± 16.2	c 100.6 ± 7.7	c 39.7 ± 22.5	d 27.6 ± 3.4
	Subjected	a 380.6 ± 21.3	a 267.2 ± 42	a 179.7 ± 16.2	a 152.6 ± 29.2	a 53.2 ± 11
40 – 45	Non subjected	d 162.8 ± 11.7	d 143.8 ± 17.2	d 59.5 ± 10	b 77.3 ± 10.2	d 28.6 ± 3.3
	Subjected	c 297.1 ± 28.3	c 185.6 ± 6.9	c 99.7 ± 12.3	a 159.2 ± 39.6	c 35.5 ± 2.5
LSD		27.3	38.4	16.1	21.6	6.7

Conclusion

beside a significant decline in high density lipoprotein (HDL) of all Subjected samples as compared with the Non subjected ones at ($P \leq 0.05$); effects on redox cycles and peroxides production and hence the radiofrequencies cause the elevations of AST and ALT, also The elevations of blood calcium and potassium could result also by the frequency exposure as that these ions of great equilibrium in intracellular and extracellular compartments.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Agarwal A, Deepinder F, Sharma RK, Ranga G, Li J. Effect of cell phone usage on semen analysis in men attending infertility clinic: an observational study. American Society for Reproductive Medicine. 2008; 89(1).
2. Aweda MA, Gbenebitse S, Meindinyo R O. Microwave radiation exposures affect the ldl, hdl, tcl and trg status in rats. International Journal of the Physical Sciences. 2010; 5(7): 1015-1022.
3. Nageswari KS. Biological Effects of Microwaves and Mobile Telephony. Proceedings of the International Conference on Non-Ionizing Radiation at UNITEN (ICNIR) Electromagnetic Fields and Our Health 20th–22nd October. 2003.
4. Yaghmaei P, Parivar K, Doranian D, Hashemi M, Torkaman F. Study the effect of extremely low frequency electromagnetic fields on some blood serum's lipoproteins, liver enzymes and P448/ P450cytochrome enzyme system in NMRI female mice. Journal of Paramedical Sciences (JPS). 2010; 1(1): 46-52.
5. Blackman CF. ELF effects on calcium homeostasis. In: Wilson BW, Stevens RG, Anderson LE (eds) Extremely Low Frequency Electromagnetic Fields: the Question of Cancer. Battelle Press, Columbus, Ohio. 1990; 189-208.
6. Dindic B, Sokolovic D, Krstic D, Petkovic D, Jovanovic J, Muratovic M. Biochemical and Histopathological Effects of Mobilephone Exposure on Rat Hepatocytes and Brain. Acta Medica Medianae. 2010; 49(1):37-42.
7. Naito H, Kaplan A. Cholesterol. Clin Chem. the C.V. Mosby Co. St. Louis. Toronto. Princeton. 1984; 1194-11206.
8. Friedewald W, Levy R, Fredrickson D. Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge. Clin. Chem. 1972; 18(6): 499-502.
9. Tietz NW. Fundamentals of Clinical Chemistry. WB, Saunders Co., Phila, PA, 2nd Ed. 1976; 876.
10. Tietz NW. (1999). Text book of clinical chemistry, 3rd Ed. C.A. Burtis. E.R. Ashwood. W.B. Saunders. 1999; 819-861.
11. Fossati P, Prencipe L. Clin. Chem. 1982; 28: 2077-2080.
12. Stern J, Lewis WHP. Clin. Chim. Acta 2. 1957; 576.
13. Young DS. Effects of diseases on Clinical Lab. Tests. 2001. 4th Ed.
14. Reitman S, Frankel S. Am. J. Clin. Pth. 1957.
15. McCrindle BW. Screening and management of hyperlipidemia in children. Pediatr. Ann. 2000; 29: 500-508.
16. Weijenberg MP, Feskens EJ, Kromhout D. Total and high density lipoprotein cholesterol as risk factors for coronary heart disease in elderly men during 5 years of follow-up. Circulation. 2009; 119: 1108-1115.
17. Al-Uboody WSH. Effect of mobile phone electromagnetic waves on the haematological and biochemical parameters in laboratory mice (*Mus musculus*). Bas.J.Vet.Res. 2015; 14(2): 250-264.
18. Senturk HBC, Hatemi I. A clinical approach to high levels of liver enzymes. Cerrahpasa Medicine Faculty. Clin. Gastroentol. Symp. Series. 2004; 38: 9-13.
19. Garba IH, Gregory U. Serum Alkaline Phosphatase activity as a potential biomarker for the intergrity of the hepatic drainage system in acute falciparum malaria infection. Internet. J. Infect. Dis. 2005; 4: 1-5.
20. Al-Uboody WSH. Effects of Electromagnetic Waves of Mobile Phone Towers on Lipid profile,

Liver functions and Blood Electrolytes of Human Beings. International Journal of Advanced Research. 2015; 3(6): 1302-1308.

21. Goldsworthy A. Effects of electrical and electromagnetic fields on plants and related topics. In: Volkov AG (ed.) Plant Electrophysiology – Theory & Methods. Springer-Verlag Berlin Heidelberg. 2006; 247-267.
22. Dicarlo AL, White NC, Litovitz TA. Mechanical and electromagnetic induction of protection against oxidative stress. Bioelectrochemistry. 2000; 53: 87-95.27.
23. Al-Uboody WSH, Waad SK, Abbas MK. Sequelae of Recurrent Impact with Radiofrequency in Mice. Indian Journal of Public Health Research and Development. 2018; 9(10): 860-865.

Assessment of Transdermal Vasodilatatory Effect of a Combined Panthenol, Amlodipine, Isosorbide Dinitrate and Betahistine HCl on Peripheral Vessels

Hussein Abdulkadhim A[1], Naser A Naser[2], Fadhaa A. Ghafil[3], Rana Talib Al-Nafakh[4], Karrar K. Alhaidary[4]

[1]Assisty Prof. dr. Department of Pharmacology, Faculty of Medicine, [2]Assisit Prof. dr. Department of Chemistry, Faculty of Science, [3]Lecturer, [4]Assist Lecturer: Department of Pharmacology, Faculty of Medicine, University of Kufa, P.O. Box(21), Najaf Governorate, Iraq

Abstract

Peripheral vascular diseases are group of disorders characterized by stenozing peripheral circulation as a complication of primary disease like diabetes mellitus. Peripheral vascular diseases affect 202 million around the world. Peripheral vascular complications of diabetes mellitus are common which affect 30 million over the world and may give rise to infectious necrotizing sequalae called diabetic foot. In a trial of assessment for transdermally applied vasodilator drugs, 30 individual had participated in a case controlled study. Test group (N =11) and control group (N =19) were assessed for the signs of vasodilatation over the dorsum of the hand and figure blood perfusion detection. There was significant increase in perfusion index (from 7 to 11) induced by the test vasodilator as compared with control (from 7 to 8) $P < 0.05$ and parallel results were obtained in induction of redness (redness ratio of 7 with C.I. over 0.95 and thermal increase in degrees of (C) over the dorsum of the hand in comparison with the control group. From the overall results the combined vasodilatatory transdermal formula caused a significant peripheral vasodilatation which could be a candidate therapeutic effect in diabetic foot.

Key words: *PVD; combined transdermal formula; peripheral blood perfusion; thermal effect; nitrate; RGB.*

Introduction

Peripheral vascular disease (PVD) is the abnormality of vessels located outside of the heart and brain, mainly leg vessels characterized by complete or partial blockage and impairment of perfusion. PVD could affect both arteries (peripheral arterial disease PAD) and veins. PVD is one of the significant health challenges that affects up to 20% after the age of 60 year [(1,2)] and 202 million patients around the world [(3,4)].

Different etiologic and pathogenic factors might share common impacts on peripheral circulation. Of the most critical form of PVD presentation is the angiopathic complications of diabetes mellitus[(5-7)].

However, Burger disease and Raynaud phenomenon are also characterized by impaired peripheral circulation [(8,9)]. Impaired peripheral circulation in uncontrolled diabetes is characterized by different pathological processes including occlusive ischemia [(10-12)], impaired immune response and peripheral neuropathy [(13,14)]. Impairment of circulation and immune system in addition to hyperglycemia and neuropathy can severely deteriorate any skin lesion in the foot. That lesion may be refractory to treatment due to lacking of pharmacokinetic opportunity of drugs treatment to diffuse to extreme tissues of the lower limbs. This pathogenic fact make foot prone for untreatable infections that even cause ascending cellulitis, tissue gangrene and septicemia which is frequently necessates amputation of the foot or lower limb[(15)].

A potent systemic vasodilatatory approach like nefidipine, nicorandil can cause intolerable adverse effects like palpitation, throbbing headache and edema due to the need for extensive vasodilatation [(16-18)]. Transdermal route of application gives opportunity for rational option of applying combined efficacious

vasodilators just proximal to the site of lesion that bypassing systemic impacts [19-21].

Different methods are used to assess peripheral circulation. These include: Doppler ultrasound, angiographic techniques in addition to the clinical assessment of peripheral pulsation. However, other methods like infrared detection of blood flow. Quantitative assessment of peripheral perfusion is detected by the perfusion index PI which ranges between 0.02% to 20% according to site whereas blood flow is 5000 ml/min[22,23]. Tissue blood flow rate varies also according to type of tissue. Thermographic camera images are also reliable noninvasive methods for long term follow up of circulatory perfusion [24-26].

In the present work, the effect of transdermally applied combination of panthenol, mineral oil base, amlodipine, pH buffer, isosorbide dinitrate, stabilizer and betahistine with peripheral perfusion rate and thermographic analysis were assessed.

Sample, Materials and Method

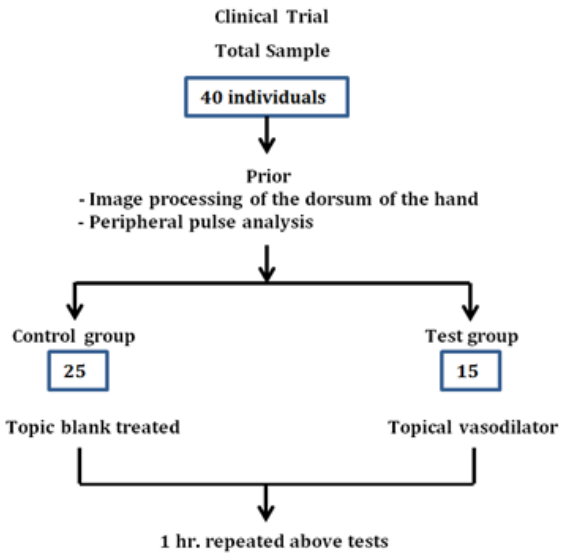


Figure (1) A diagram of the main practical steps of conducting this study.

A 40 individuals (20f/20m) aged 20-24 years (body weight 50-65 kg) have participated in clinical assessment of the effects for the designed formula.

Groups of the study:

1- Test group (N = 15: 8 f/ 7 m).

2- Control group (N = 25: 12 f/ 13 m).

All persons had no chronic medical illnesses.

Study design

The study was arranged in a pilot clinical trial design to determine the effects of test formula so that one group had transdermally applied vasodilators and the other had only the blank application.

Medical and Research ethics requisites

These considerations were fulfilling Geneva requisites guide for medical ethics. All of the participants were informed about the study design and the expected topical adverse effects of the agents used.

Free autonomy, drugs safety, rights reservation, individual consents and drugs benefits were considered and insured.

Materials used

All the materials used were processed, filtered, and confirmed with Fourier UV/V range 200-700 nm based on British Pharmacopia 2007 drugs standard spectrophotometry.

Drug dosages were weighed for base of transdermal formula design.

- Amlodipine (Bristo, UK, production vs expiry:PD-ED: 2013-2016)

7000 mg package. Used in 3% within transdermal design.

- Isosorbide dinitrate (Epico, Egypt, PD-ED: 2013-2015)

100 mg package. Used in 0.05%.

- Panthenol (Zynova, Oman, PD-ED: 2014-15). Used in dosage of 0.3%.

- Betahistine HCL (Aleppo, Syria, PD-ED; 2013-2015). Used in 0.02%.

- Hydrocarbone base vehicle.

Mineral laurate, stearate and solid excipients were prepared for 20 ml per dosage form.

Treatment Mode

Objective vasodilatation parameters were measured prior to application of both blank and test formula as a

zero reading. Then 10 minutes after application of the blank and the control formula another record of RGB, thermograph and PI were measured after careful topical message over the dorsum of the right hands only for standardization.

Methods of assessment of vasodilatation

Peripheral blood perfusion was measured with Beijing Safe Heart Technology. Transducer was connected to PERFUSION-Kufa program of analysis developed by Dr. Hussein Abdulkadhim on Mathwork 2013a blockset for estimating model formula of perfusion in response to the transdermal test treatment.

Perfusion index PI is readily detected and calculated by the computer from which another measurement could be estimated which is the perfusion range is determined with in unit of time to calculate the rate of perfusion in ml/min and compared for the control and observational control group. The fixed level of the hand and index figure was carefully considered because it is important confounder.

Another assessment method for vasodilatatory activity of the test formula includes thermographic correlative analysis detected by combined spot tissue thermal camera

Since temperature correlates proportionally with rate of peripheral blood perfusion, a rise in temperature of the dorsum of the hand correlates with a parallel rise in the rate of perfusion (parameters detailed in guidelines manual).

Image processing program was used to analyze RGB shift as an indicator of redness associated with vasodilatation by the test combination.

Lab techniques quality confirmation:

Accuracy of Safe Heart Technology was X +/- 0.2 PI. Efficiency of the thermographic camera was insured by software processing and calibration.

UV/V (Cecil, UK, Programmed wavelength, accuracy: X +/- 0.5%). Sensitive Balance (mini digital, China, accuracy: X +/- 0.01 mg). PHELECT, USA computerized pH measuring electrode is used to assess adjustment of formula pH around 7.5.

The Sample Size, Statistical Processing and Analysis

The sample size for this study was calculated based on the Cochran formula n= z^2 * p*q/e^2 where (e) is the margin error, p and q are the complimentary proportions and z is the score at confidence level 95% equals to 1.96. Although the margin error will be wide, however it's preferred to reduce the sample size as much as it's statistically possible in a pilot clinical trial in order to fulfill FDA approval guidelines where a sample size of 10 individuals is considered while conducting a phase zero trial.

Mathwork 2013a model interpolation was used to verify the perfusion curve. Perfustion index PI modification ratio was estimated with C.I. of 0.24 and a cnfidence level of 95%. Sample size was determined based on Cochran formula. Paired t test with Minitab 2014 at P <0.05.

Results

Calculation of the rate of perfusion.

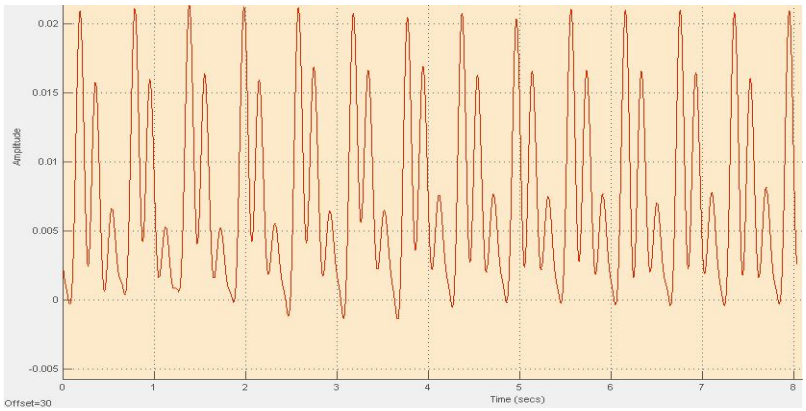


Figure (2): Matlab analysis of peripheral perfusion rate by model analysis and rate determination. The amplitude axis was calibrated to obtain perfusion rate in mL/min.

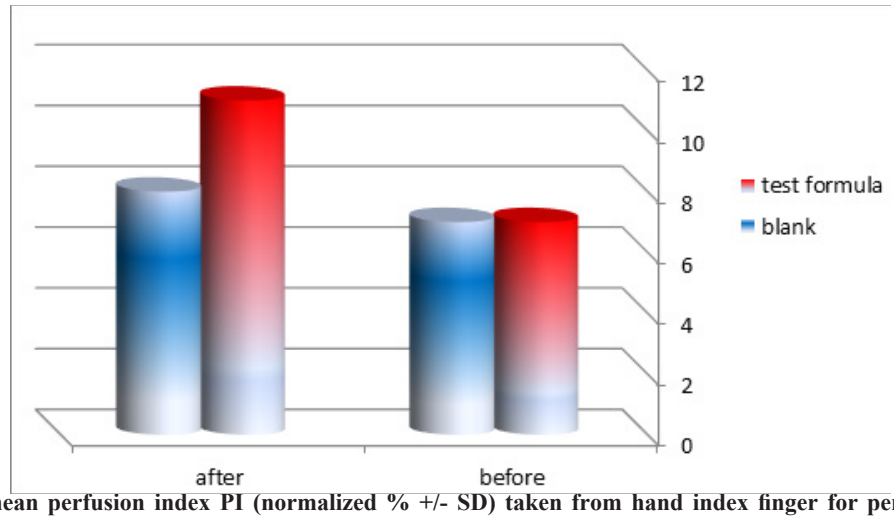
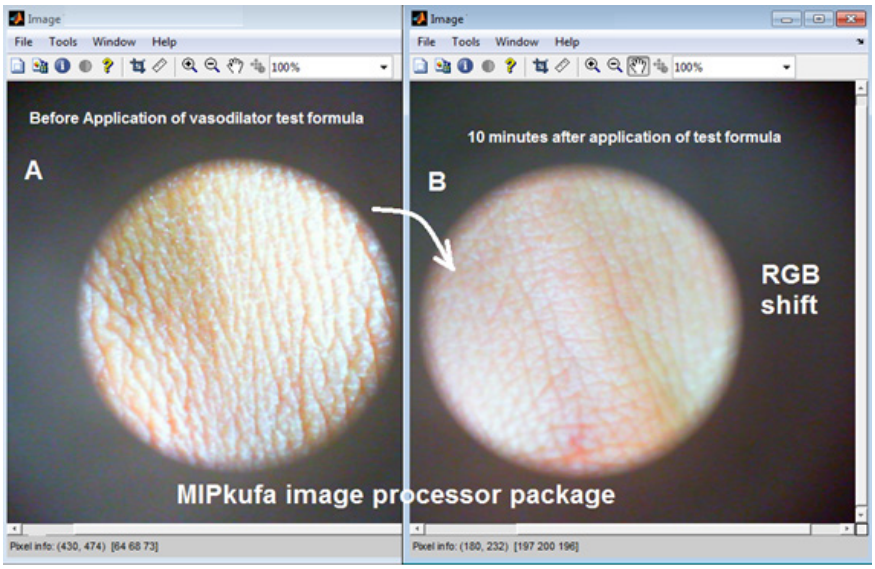


Figure (3): The mean perfusion index PI (normalized % +/- SD) taken from hand index finger for persons taking the test transdermal formula (raised from 7 +/- 2 to 11 +/- 2) as compared with the control (7 +/- 2 to 8 +/- 2 PI).



Topical RGB findings for assessment of redness induction as an indicator of vasodilatatory effect.

Figure (4): The objectively analyzed RGB to determine level of redness induced on the dorsum of the hand by the combined vasodilator formula after 10 minutes from topically being applied as a quantitative indicator of vasodilatatory effect.

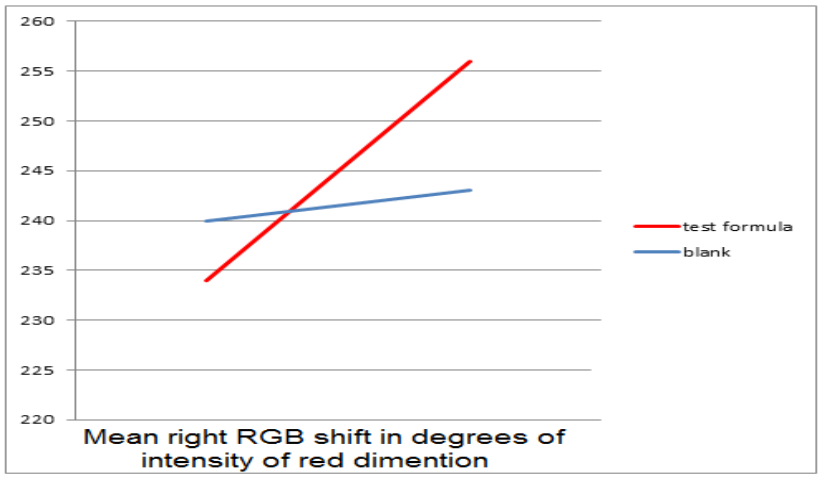


Figure (5): The effect of the test formula on the RGB right shift of red intensity in comparison with the control. Red intensity shift was from 234 to 256 as compared to control which caused just a shift from 240 to 243.

Discussion

The sample of this study represented healthy nondiabetic population. It was taken as a model to assess the vasodilatation at the upper extremities where temperature, RGB and peripheral perfusion were measured from.

That sample was beneficial to estimate the vasodilatatory prior to recommendation in diabetic patients' sample since this was in agreement with FDA phases of drug evaluation.

Transdermal approach is a promising way of treatment since many advanced techniques had made this route more reliable and superior to conventional systemic administration of drugs. Of these advance techniques are the nanoparticulate reservoir of active drug, sonophoresis, electrophoresis, microarray needle and matrix patches [27].

Transdermal application of drugs has many advantages like convenience, safety and it is easily controlled.

The results showed that a significant increase in perfusion index (PI) was obtained with the test formula (from 7 to 11%) in comparison with the blank base (from 7 to 8%) with ratio of increase 1.3 in PI. In one study, amlodipine showed a significant induction of increased forearm blood flow. Results of some studies concerned assessment of vasoliatatory effect of transdermal amlodipine monotherapy have agreed with this current study [28]. Panthenol in combination with other additives was used as a topical wounds healing enhancer [29]. Betahistine effects in some applications agreed with this study [30], however there was no improvement of cold regional pain syndrome by using transdermal isosorbide dinitrate in a small controlled trial [31,32].

Redness parameters have confirmed and went parallel to data of perfusion rate increment. The mean RGB was raised from 234 to 256 in test formula group as compared with a mild raise from 240 to 243 in blank taken group. This indicates a redness ratio of 7 with C.I. at 0.95 [33].

Thermographic results revealed parallel findings with redness image processing outcomes. Hand temperature was raised from 36.0 to 37.8 °C in vasodilatatory test group in comparison with 35.5 to 36.1 °C in the control. The temperature raising activity was 3 and C.I. at 0.95.

Conclusion

From the overall effects, the combined transdermal formula panthenol, mineral oil base, amlodipine, pH buffer, isosorbide dinitrate, stabilizer and betahistine showed a significant vasodilatatory effects on the peripheral circulation. There was a significant increase of thermal effect due to the topical combination of vasodilators in comparison with the control

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1- Malahlela P, Motswaledi M. Management of mild to moderate acne vulgaris. South African Family Practice. 2013; 55(3): 241-244.

2- Sartorius K, Boer J, Gregor B, Jemec J. Topical Treatment. Hidradenitis Suppurativa. 2006; 20(3): 150-160 .

3- Decker A, Graber EM. Over-the-counter Acne Treatments: A Review. J Clin Aesthet Dermatol. 2012; 5(5): 32-40.

4- Webster GF, Graber EM. Antibiotic Treatment for Acne Vulgaris. Semin Cutan Med Surg. 2008; 27(3):183-187.

5- Peter T. Riis, Hans C. Ring, Lotte Themstrup, Gregor B. Jemec. The Role of Androgens and Estrogens in Hidradenitis Suppurativa – A Systematic Review. Acta Dermatovenerol Croat. 2016 ; 24(4): 239-249.

6- Chandersekar L. Review on Pathophysiology and Treatment of Acne. RJPBCS. 2013; 4(2): 1355-1361.

7- Jude EB, Eleftheriadou I, Tentolouris N. Peripheral arterial disease in diabetes–A review. *Diab Med.* 2010; 27(3): 4-6.

8- Tanghetti EA. The Role of Inflammation in the Pathology of Acne. The Journal of Clinincal and Asthetic Dermatology. 2013; 6(9):27-35.

9- Maria GV, Bjarne RR, Lene Moeller SM, Anne GP. Complex regional pain syndrome. Musculoskeletal

Science & Practice. 2016; 26(9): 223–230.

10- Ivan S, Galstyan GR, Solomatina KY, Bondarenko ON, Mitish VA, Doronina LP. Critical limb ischemia and diabetic foot treatment in a multidisciplinary team setting. Cardiac & Vascular Update. 2012; 2(11): 24-29.

11- David CH, David GA. The Multidisciplinary Team Approach To The Diabetic Foot. *Podiatry Today*. 2016; 29(6): 16-21.

12- Caitlin WH, Joseph KC, Hikmet K, Nestoras M, Ronald LS, James HB, Christopher JA. The Society for Vascular Surgery Wound, Ischemia, and foot Infection (WIfI) classification system correlates with cost of care for diabetic foot ulcers treated in a multidisciplinary setting. J of Vascular Surgery. 2018; 67(5): 1455–1462.

13- Fowkes F., Leng GC. Bypass surgery for chronic limb ischemia. Cochrane Database Syst Rev. 2008; 16(4): 188-200.

14- Antoniou GA, Georgiadis GS, Antoniou SA, Makar RR, Smout JD, Torella F. Bypass surgery for chronic lower limb ischaemia. Cochrane Database of Systematic Reviews. 2017 ; 34(7): 345-357.

15- Jude R, Nivedita M. Diabetic Foot and Gangrene. Current Concepts and Management Options. 2011; 17(2): 123-144.

16- Strauss JS, Krowchuk DP, Leyden JJ, Lucky AW, Shalita AR, Sigfried EC. Guidelines of care for acne vulgaris management. J Am Acad Dermatol. 2007; 56(4): 651-63.

17- Zaenglein A, Pathy A, Schlosser B, Alikhan A, Baldwin H, Berson D. Guidelines of care for the management of acne vulgaris. J Am Acad Dermatol. 2016; 74(5): 945-973.e33.

18- Anthony J. Incidence, prevalence and pathophysiology of acne. Adv. Stud.Med.2008; 8(4):100-105.

19- Leave AC, Nuno M, Christiane D, Catarina PR. Recent Advances in Drug Delivery Systems. Scientific Research. 2011; 2(5): 510-526.

20- Kinam P. The Controlled Drug Delivery Systems: Past Forward and Future Back. J Control Release. 2014 ; 190(23): 3–8.

21- Stephen M, Ipsita R. Advances in Drug Delivery. Journal of Chemical Technology & Biotechnology. 2015; 90(7): 1167-1168.

22- Hager H, Reddy D, Kurz A. Perfusion Index - A Valuable Tool To Assess Changes in Peripheral Perfusion Caused by Sevoflurane. Anesthesiology 2003; 99(A):593-599.

23- Michał K, Anna F, Andrzej N. New-generation pulse oximetry for the assessment of peripheral perfusion during general anaesthesia — a comparison between propofol and desflurane. Anaesthesiol Intensive Ther. 2013; 45(3): 138–144.

24- Friedlander SF, Eichenfield LF, Fowler JF, Fried RG, Levy ML, Webster GF. Acne epidemiology and pathophysiology. Semin Cutan Med Surg. 2010; 29(21): 2-4.

25- Bhate K, Williams HC. Epidemiology of acne vulgaris. British Association of Dermatologists. 2013;168, 474–485.

26- Soodabeh Z; Behrouz V; Hamid A. Determination of microbial agents of acne vulgaris and Propionibacterium acnes antibiotic resistance in patients referred to dermatology clinics in Kerman, Iran. Jundishapur Journal of Microbiology. 2011; 4(1): 17-22.

27- Archana K. Transdermal drug delivery system: Formulation aspects and evaluation. Comprehensive Journal of Pharmaceutical Sciences. 2013; 1(1): 1–10.

28- Lincy J, Arun K, Sandra S. Formulation and evaluation of amlodipine transdermal patches using ethyl cellulose. International Research Journal of Pharmacy. 2013; 4(10): 84-87.

29- Bilgili M, Yildiz H, Sarici G. Prevalence of skin diseases in a dermatology outpatient clinic in Turkey. A cross-sectional, retrospective study. Journal of Dermatol Case Report. 2013; 7(4):108-120.

30- Josef M, Colleen L, Jochen S, Diane P. Composition and Method of Treating Hearing Loss. United States Patent. 2011; 7(B2): 945-951.

31- Groeneweg JG, Huygen FJ, Niehof SP, Wesseldijk F, Bussmann JB, Schasfoort FC. No Recovery of Cold Complex Regional Pain Syndrome After Transdermal Isosorbide Dinitrate: A Small Controlled Trial. Journal of Pain and Symptom Management. 2009; 38(3): 401-408.

32- Silvia D, Daniela B, Keith JT, Roderick JF. Treatment of complex regional pain syndrome: an

updated systematic review and narrative synthesis. Can J Anesth/J Can Anesth. 2018; 65(1): 658–684.

33- Zhao WJ, Duan F, Li ZT, Yang HJ, Huang Q, Wu KL. Evaluation of regional bulbar redness using an image-based objective method. International Journal of Ophthalmology. 2014;7(1): 71-76.

Evaluation of Using Titanium Mesh in the Reconstruction of Traumatic Orbital Floor Fracture

Mukhalled Salim Alasady[1], Ihsan Abdullah Kumail [1], Alyaa Turky Alsultany[2], Ali B. Roomi[3]

[1] Department of Oral and Maxillofacial Surgery, College of dentistry, Al-Ayen university, Thi-Qar, Iraq, [2]Directorate of Education Al-Muthanna, Al-Muthanna, Thi-Qar, Iraq, [3]College of Health and Medical Technology, University of Al-Ayen, Thi-Qar, Iraq

Abstract

Background: Among the nonresorbable implants, Titanium mesh is the most common graft used for orbital reconstruction. It is continuously improved to achieve accurate restoration of orbital volume. To evaluate the using of Titanium mesh for the reconstruction of traumatic orbital floor fractures.

Patients and Methods: A total of (32**)** patients were enrolled in this study where operated under general anesthesia to repair the orbital floor fractures by using titanium mesh, and they were (24) males and **(**8) females. (16) patients had Enophthalmos,(10) patients had Diplopia,(6) patients had both enophalmos with diplopia; all patients had ecchymosis, Subconjunctival hemorrhage, Parasthesia of infraorbital nerve.

Results: The results were well represented as the following: (29)patients(90.62%) had no both diplopia & enophthalmos. Postoperative complication had been found in only(3) patients (9.3%), Two patients(6.25%) had diplopia, one patient (3.1%) had Enophthalmos.

Conclusions: Titanium mesh has a long track record of reconstruction of large orbital floor defects and correction of globe malposition.

Keywords: *Titanium mesh; orbital reconstruction and fracture*

Introduction

Orbital floor fractures have specific clinical attention for many reasons. Failure to recognize and treat them early may result in severe sequelae. However, despite surgical intervention, orbital floor fractures are associated with the risk of persisting sensibility disorders, enophthalmos, and permanent diplopia. [1] The choice of the ideal material for reconstruction of orbital floor and walls remains highly controversial. Many materials, from different sources, have been described for that task. The ultimate goals are the reconstruction of the bony orbital defect with restoration of anatomy, volume, function, and esthetics. Each type of material has advantages and disadvantages, but the most important characteristic of a material is to allow those surgical objectives to be achieved. [2] Titanium mesh is the most common grafts used for orbital reconstruction because of its biocompatibility, availability, rigid fixation and no donor site needed. [3] There is still controversy existing about the ideal material for orbital reconstruction; autogenous and synthetic materials have been used for many years, and both of them have merits and demerits. The common sources of autogenous graft are the calvarium [4], rib, iliac crest, and auricular or nasal septal cartilage. Sakakibara et al. [5] used the iliac cancellous bone of only 1 mm thickness for the reconstruction of the orbital floor. Numerous synthetic materials have been developed with the advantages of availability, no donor site morbidity, and decreased operation time. The choice of material for reconstruction is largely determined by the experience of the surgeon and implant cost. Among the nonresorbable implants, titanium mesh and Medpor [6] are the most common grafts used for orbital reconstruction. Both materials are being

Corresponding author:
Ali B. Roomi
E-mail: dr.ali_bader@alayen.edu.iq,
alibadr205@gmail.com

continuously improved to achieve accurate restoration of orbital volume. During the past several decades, the standard of care for orbital reconstruction after trauma has been autogenous bone grafts, complications of bone grafts, including donor site morbidities such as scar alopecia and graft resorption with delayed enophthalmos, have inspired an interest in the use of alloplastic substitutes such as titanium, Titanium‟s role in orbital reconstruction was limited originally to small orbital defects, and as an adjunct to bone grafts, more recently, clinical studies have documented the sole use of titanium mesh to reconstruct large orbital defects. (7) Orbits reconstructed with titanium mesh show better results than those reconstructed with bone grafts. (8-10)

Titanium is a chemical element with symbol Ti and atomic number 22. It is a lustrous transition metal with a silver colour, low density, and high strength. (11) Titanium can be alloyed with iron, aluminium, vanadium, and molybdenum, among other elements, to produce strong, lightweight alloys. (11) The two most useful properties of the metal are corrosion resistance and the highest strength-to-density ratio of any metallic element. (12) In its unalloyed condition, titanium is as strong as some steels, but less dense.(12) Titanium is used in steel as an alloying element (ferro-titanium) to reduce grain size and as a deoxidizer, and in stainless steel to reduce carbon content. (11) Titanium is often alloyed with aluminum (to refine grain size), vanadium, copper (to harden), iron, manganese, molybdenum, and other metals. (13)

Medical applications: Because it is biocompatible (it is non-toxic and is not rejected by the body), titanium has many medical uses, including surgical implements and implants, such as hip balls and sockets (joint replacement) that can stay in place for up to 20 years. (13) The titanium is often alloyed with about 4% aluminum or 6% Al and 4% vanadium. (14) Because titanium is non-ferromagnetic, patients with titanium implants can be safely examined with magnetic resonance imaging (convenient for long-term implants). The features of 3D Titanium mesh are:malleable,0.3mm thickness,1-1.3mm screws,available for right and left sides. (15) Advantages of titanium mesh plates :(15)

1. availability, biocompatibility
2. ease of intraoperative contouring
3. rigid fixation
4. .Radiopacity
5. No donor site needed
6. Tissue incorporation may occur

Disadvantages:

1. Irregular edges of the mesh may catch prolapsed orbital fat. (16)
2. .difficulties with ease of insertion
3. difficult to remove if required

Complications of titanium mesh:

1. While a nonresorbable material, titanium cannot be replaced by new soft tissue or bone tissue and remains in situ indefinitely. This may cause possible late side effects, including toxicity due to metal ion release. (17)

2. The fibrous reaction between the implant and the orbital contents caused the eye movement restriction and the lid retraction. (18)

3. To avoid adherence syndrome, titanium mesh plates should be placed 2 mm away from the orbital rim. (18)

Patients and Methods

A total of (32) patients were enrolled in this prospective study was conducted from 2014 to 2016. There were (24) males and (8)females. Patients' age ranged between (10- 50) years. The mean age was 30 years. **Inclusion criteria:** Patients were included in this study according to the following criteria: Patients with orbital floor fractures regardless of their age or gender and type of missile injury (Blast or Bullet), Orbital floor defect(small to very large defects) confirmed by C.T. scanning, Patients with Enophthalmos and Patients with Diplopia due to mechanical obstruction. **Exclusion criteria:** Patients with the following criteria were excluded from the study: Serious general disease and unfit for surgery, Refused to participate, All orbital fractures regardless of the fracture site and Patient with diplopia due to neural causes.

Measuring of Enophalmos:

The mean preoperative enophthalmos was about (4) mm measured by a ruler from the lateral orbital rim of the injured eye to the most anterior projection of the

globe in comparison to the intact contra-lateral eye.

Results

Sex and age of the patients:

There were (24) males (75%) and (8) females (25%) with a male to female ratio of (3:1), (Figure 1 and 2). The age distribution of the patients revealed a mean age of(30 ± 2.1) years furthermore, {**2**} patients (6.25%) aged (10-19) years, {**10**} patients (31.25%) aged (20-29) years, **{8}** patients (25%) aged (30-36) years, **{7** } patients (21.875%)aged (37-45) years, **{5**} patients (15.625%)aged (46-50) years.

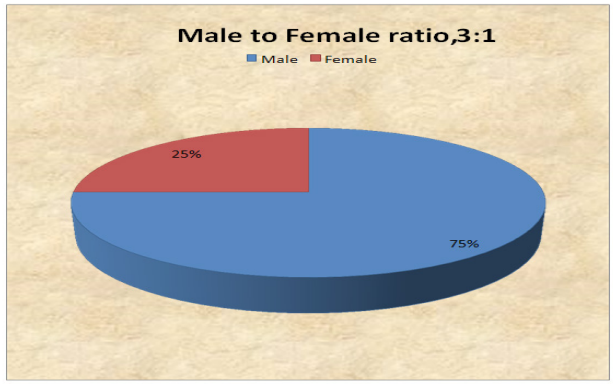


Figure 1. Gender distribution of the patients, (N=32, Male to female ratio; 3:1) **Figure 2. Age distribution as percentages of the studied group, (N=32)**

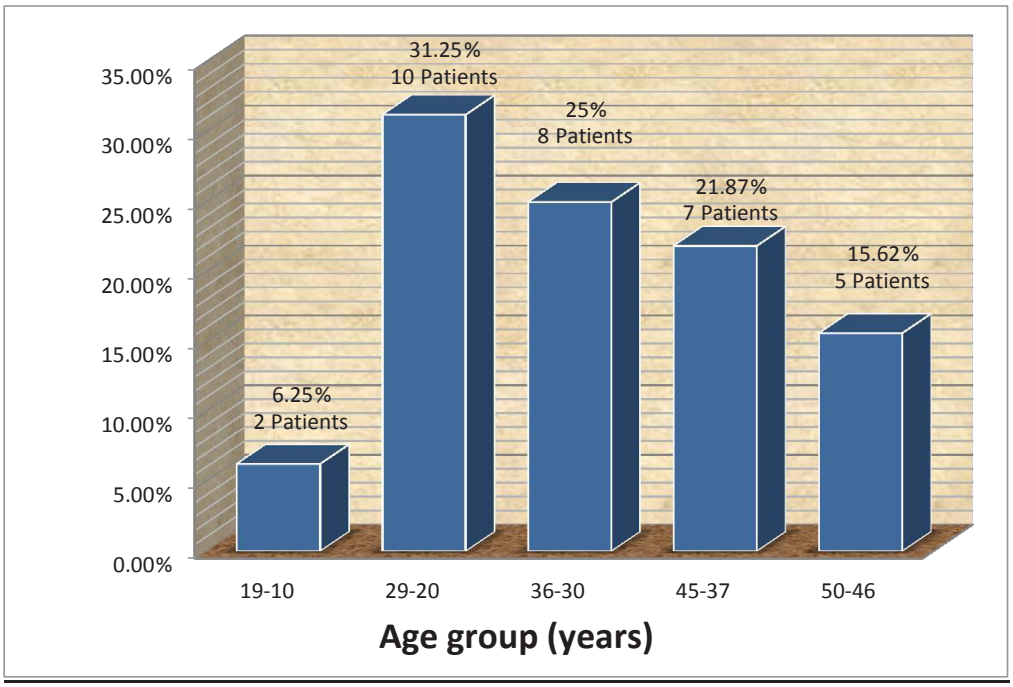


Figure 2. Age distribution as percentages of the studied group, (N=32)

Postoperative Complications

As shown in table 1, fortunately.

1. **(29) patients (90.625%) had no complications,**
2. **Post-operative complication had been found in only(3) patients (9.375%).**
3. **Two patients (6.25%) had diplopia, 3. one patient (3.125%) had Enophthalmos and**
4. **No patient (0%) had extrusion.**

Table 1. Post-operative complications of patients.

Complication	No. of patients	%
Diplopia	2	6.25
Enophthalmos	1	3.125
Extrusion of titanium mesh	0	0
No complications	29	90.625
Total	32	100

Discussion

Age Distribution

In the current study, the highest risk group weres the young patient (20-29 years) represented as(31.25%) and this was in agreement with Leibsohn et al., 1976 [19]; Greenwald et al. 1979 [20]; Crumley et al. 1977 [21]; Andersen et al. 1985 [22]. In fact that this age group represents the time of maximum activity in human life, especially males.

Sex Distribution:

Twenty four patients (75%) were males, while 8 patients (25%) were females. The predominant male to female ratio(3:1) can be explained by the fact that males spend most of their time outdoors, and due to occupational and recreational preferences. This result is consistent with the findings of the studies by: Thomas 2005 [23], Michael 2000 [24] ,David 2004 [25] ,Gordon 2004 [26], ,Joe 2005 [27]), Geoffrey 2005 [28] ,Petrus 2006 [29].

Reconstruction material:

The orbital wall is one of the most frequently damaged parts of the maxillofacial skeleton after midfacial trauma. Regardless of the fracture site, blow-out fractures can cause various functional and aesthetic sequelae. Preventing these complications from becoming long-term problems is very important, and it depends strongly on the materials used for bridging the orbital wall defects. The prerequisites of an ideal material are good biocompatibility, easy to manipulate and insertion, and it should allow fixation to the host bone by screws, wire, or sutures. It should be cheap, readily available, and strong mechanical strength to support the orbital structure. Titanium mesh has a long track record in the reconstruction of large orbital defects and correction of globe malposition. The advantages of titanium mesh plates are availability, easy intraoperative contouring, and rigid fixation. According to our work, we found it can be adapted to complex structures easily, and it can also be cut to shape as well. The orbits reconstructed with titanium mesh showed better overall reconstructions than those reconstructed with bone grafts, and according to our work, we agree with Ellis E 3rd[1], Tan Y 2003 [8].

Complications

Pre-operatively enophthalmos occur in (16) patients(50%), diplopia occur in (10) patients(31.25%), and (6) patients(18.75%) had both enophthalmos and diplopia. After reconstruction, we have only **one patient** who has persistent enophthalmos presented with extensive injury to surrounding bony structures with loss of bony architecture. We agree with the finding of { **Saikrishna Degala**, **2012** (30)}.

Postoperatively, diplopia occurs in two patients, mainly in the upward gaze. This consistent with that reported by Amrith S et al. 2000 [31].Where they reported that diplopia is not uncommon postoperatively, it is typically only disturbing when occurring in the primary or downward gaze. However, titanium plates are permanent foreign bodies. Several late-onset complications related to the titanium mesh plate have been reported, such as infection, extrusion, implant migration, residual diplopia, etc. [32]. But we found in our study, the postoperative clinical and radiographical examination verified the anatomical reduction of the orbital floor. There was no displacement or resorption of the orbital floor or loosening or extrusion of the screws or mesh (6-9) months after the operation, and no modifications in the visual acuity compared with autologous materials have several disadvantages, including high risk of nerve and blood vessel injury, donor site morbidity, cosmetic disturbance, minimal controllability and an unpredictable degree of absorption [33,34].

Conclusions

1. Missile trauma was the most frequent cause of orbital injury followed by road traffic accidents, sports injury, and falls from height.

2. Titanium mesh has less complication postoperatively, such as infection and migration of mesh.

3. This study highlights the ability of the

alloplastic mesh to satisfactorily correct post-traumatic orbital sequelae, including enopthalmos and diplopia.

4. Titanium mesh can be considered to be the excellent orbital floor repair material.

5. No extrusion of Titanium mesh or rejection by the host was seen in the studied group.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Raghoebar GM, Brouwer TJ, Reintsema H, Van Oort RP: Augmentation of the maxillary sinus floor with autogenous bone for the placement of endosseous implants: a preliminary report. J Oral Maxillofac Surg 1993; 51(11): 1198-1203.
2. Ellis E III, Messo E. Use of nonresorbable alloplastic implants for internal orbital reconstruction. J Oral Maxillofac Surg 2004;62:873–881
3. Ochs M.W. (2004): Orbital and ocular trauma: In Michael Miloro editor: Peterson's principles of the oral and maxillofacial surgery second edition. BC Decker, Inc.
4. Burm, J. S., Chung, C. H., and Oh, S. J. Pure orbital blowout fracture: New concepts and importance of medial cubital blowout fracture. Plast. Reconstr. Surg. 1999; 103: 1839.
5. Patel PC, Sobota BT, Patel NM, et al. Comparison of transconjunctival versus subciliary approaches for orbital fractures: a review of 60 cases. J Craniomaxillofac Trauma 1998; 4:17–21.
6. Taban M, Nakra T, Mancini R, et al. Orbital wall fracture repair using seprafilm. Ophthal Plast Reconstr Surg 2009; 25:211–214.
7. Annals of Plastic Surgery: January 2002 - Volume 48 - Issue 1 - pp 1-9
8. Ellis III E, Tan Y – Assessment of Internal Orbital Reconstructions for Pure Blowout Fractures: Cranial Bone Grafts versus Titanium Mesh. *J Oral Maxillofac Surg* 2003;61:442-453
9. Kuttenberger JJ, Hardt N – Long-term Results Following Reconstruction of Craniofacial Defects with Titanium Micro-Mesh Systems. *J Craniomaxillofac Surg* 2001;29:75-81
10. Gear AJL, Lokeh A, Aldridge JH, et al. – Safety of Titanium Mesh for Orbital Reconstruction. *Ann Plast Surg*2002;48:901-910
11. *"Titanium". Encyclopædia Britannica. 2006. Retrieved 29 December2006.*
12. Donachie, Matthew J., Jr. (1988). *TITANIUM: A Technical Guide*. Metals Park, OH: ASM International. p. 11. ISBN 0-87170-309-2.
13. Hampel, Clifford A. (1968). *The Encyclopedia of the Chemical Elements*. Van Nostrand Reinhold. p. 738. ISBN 0-442-15598-0.
14. Orthopaedic Metal Alloys". Totaljoints.info. Retrieved 27 September 2010.
15. Ellis E 3rd, Tan YH. Assessment of internal orbital reconstructions for pure blowout fractures: cranial bone grafts versus titanium mesh J Oral Maxillofac Surg 2003;61:442Y453
16. Tuncer S, Yavuzer R, Kandal S, et al. Reconstruction of traumatic orbital floor fractures with resorbable mesh plate. J Craniofac Surg2007;18:598Y605.
17. Kozakiewicz M, Elgalal M, Loba P, et al. Clinical applications of 3D pre-bent titanium implants for orbital floor fractures. J Craniomaxillofac Surg 2009;37:229Y234.
18. Lee HB, Nunery WR. Orbital adherence syndrome secondary to titanium implant material. Ophthal Plast Reconstr Surg 2009;25:33Y36 .
19. Leibsohn J., Burton T. C. and Scott W. E. (1976): Orbital floor fractures: a retrospective study, Ann. Ophthalmol; 8:1057.
20. Greenwald H.S., Jr. Keeney, A. H., and Shannon, G.M. (1979): A review of 128 patients with orbital fractures, Am J. Ophthalmol; 78:655.
21. Crumley R. L., Leibsohn J., Krause C. J., et al. (1977): Fractures of the orbital floor; 87: 934.
22. Andersen M., Vibe P., Nielsen I.M., et al. (1985): unilateral orbital floor fractures, Scand J. Plast. Reconstr. Surg.; 19:193.
23. Thomas Widell, MD. Fractures, orbital, emedicine Journal, April, 2005. Available fromhttp://www.emedicine.com.
24. Michael E. Decherd, MD. Maxillary and Periorbital fractures. Grand Rounds Presentation, UTMB, Dept. of otolaryngology, January 26,2000 .

25. David K. Coats, MD, Evelyn A. Paysse MD, Jerane Kong MD. Unrecognized Microscopic Hyphema Masquerading as a closed Head Injury. PEDIATRICS, Official Journal of the American Academy of Pediatrics, Vol. 102, No. 3, September, 1998, PP. 652654.
26. Gordon Shields, MD. Maxillary and Periorbital Fractures. Grand Rounds Presentation UTMB, Dept. of Otolaryngology, January, 2004.
27. Joe Rabson, MD. Globe rupture. Emedicine Journal, July 13, 2005. Available from: http://www.emedicine.com.
28. Gecffrey M Kwitko, MD, FACS, FICS. Orbital fracture, floor. emedicine Journal, December9,2005.
29. Petrus Pereira Gomes DDS, MSc.,PhD., Luis August Passeri DDS, MSc, PhD and Jose Ricardo de Albergaria Barbosa DDS, MSc, PhD. A 5-year Retrospective Study of Zygomatico-orbital complex and Zygomatic Arch Fractures in Sao Paulo state, Brazil . Journal of oral and maxillofacial surgery, Vol. 64, Issue1, January 2006, P. 63-67.
30. Saikrishna Degala • Sujith Kumar Shetty • Lakshith Biddappa J. Maxillofac. Oral Surg. (Oct-Dec 2013) 12(4):418–423 DOI 10.1007/s12663-012-0444-9.
31. Amrith S, Seang MS, Thiam CL, Timothy KYL: Ophthalmic involvement in cranio-maxillofacial trauma. J. Cranio. Maxillofac. Surg. 2000; 28: 140-147.
32. Hwang K, Kim DH. Comparison of the supporting strength of a poly-L-lactic acid sheet and porous polyethylene (Medpor) for the reconstruction of orbital floor fractures. J Craniofac Surg 2010;21:847-53.
33. Ahlmann E, Patzakis M, Roidis N, et al. Comparison of anterior and posterior iliac crest bone grafts in terms of harvest-site morbidity and functional outcomes. J Bone Joint Surg Am 2002;84:716-20.
34. Takashima S, Tateishi J, Taguchi Y, et al. Creutzfeldt-Jakob disease with florid plaques after cadaveric dural graft in a Japanese woman. Lancet 1997;350:865-6.

Association of Epstein- Barr Virus (EBV) with the Development of Nasopharyngeal Carcinoma (NPC) in Western Region of Iraq: Unmatched Molecular Case-Control Study

Mothana Ali Khalil[1], Arkan Obaid Jasim Al-Essawi[2], Mohammed Tafash Dagash[3]

[1]Assistant Professor /Department of Microbiology/Virology, College of Medicine, University of Anbar, Iraq, [2]Assistant Professor /Department of Pathology, College of Medicine, University of Anbar, Iraq, [3]Assistant Professor /Department of Surgery /Thoracic Surgery, College of Medicine, University of Anbar, Iraq

Abstract

Background: Nasopharyngeal Carcinoma (NPC) is uncommon in Iraq, but its incidence is raising due to increased exposure to diverse risk factors. Many of the NPC-related risk factors are becoming more and more apparent in Iraq. The exactly risk factors for nasopharyngeal carcinoma (NPC) in Anbar province, Iraq are not known.

Objectives: To determine the association between Epstein- Barr virus (EBV) infections and other risk factors with the development NPC of Iraqi patients.

Patients and Method: Sixty-seven paraffin-embedded tissues of NPC cases, 134 normal noncancerous nasopharyngeal biopsy samples, and tonsillectomy specimens from patients with chronic hypertrophic tonsillitis as controls were enrolled in the study that was conducted between 12 January 2012 and 21 January 2019. DNA of EBV was extracted from both controls and neoplastic tissues and analyzed by PCR technique using primers specific to EBV Latent Membrane Protein-1 Oncogene (LAMP-1) for the presence of EBV. A Questioners data form for all patients and controls were filled by the researchers regarding other risk factors of NPC including the patient's age, sex, residence, radiation exposure, history of chronic rhinitis, family history of NPC, tobacco smoking, alcohol consumption, herbal medicines, tea consumption, exposure to formaldehyde and exposure to different inhalants.

Results: The following risk factors were found to be independently associated with illness: EBV (adjusted odds ratio [OR] 7.852, 95% confidence interval [CI] 2.22–27.72), herbal medicine (OR 19.051, CI 7.56–47.95) and Family history of NPC (OR 63.717, CI 6.67–607.96).

Conclusion: Combination of family history of NPC , EBV exposure and herbal medicine was a strong risk factor for NPC.

Keywords: *Epstein - Barr virus, Nasopharyngeal Carcinoma, PCR, EBV-LMP-1, other risk factor, case-control study*

Introduction

Nasopharyngeal carcinoma (NPC) is a human malignancy derived from the epithelium of the retro-nasal cavity and it is one of the most frequent head and neck cancers with elevated prevalence rates in Asia and western North Africa [1, 2]with, approximately 86691 incident cases of NPC and 50 831 NPC-related deaths in 2012 worldwide [3, 4].

Previous studies demonstrated Epstein –Barr virus (EBV) infection, genetic susceptibility, diet, chromosomal disorders, aberrant promoter hyper methylation and other genetic related factors and other environmental exposures [1, 5, 6], Cigarette smoking, alcohol consumption and Consumption of salted [1, 7, 8], Family history of NPC) **,** formaldehyde, an industrial hygienist solvents, dusts, exhaust and pesticides have been suggested to be associated with increased NPC risk [1, 9]. The present study to know the roles of EBV with

other Risk factor in development of NPC.

Materials and Method

Study design

Case-control sets were included for analysis, provided that two control was matched for each case by age, sex and general geographical location of the case's residence. A standard questionnaire for all patients and controls were filled by the researchers regarding other risk factor of NPC. Including the patient's age, sex, residence, Radiation exposure, history of chronic Rhinitis, family history of NPC, tobacco smoking with alcohol consumption, herbal medicines, Tea consumption, Exposed to formaldehyde and, exposure to different inhalants. This study was conducted in the Anbar province and its neighbor area and biopsies were collected over a 7 years period between 3 January 2012 and 1 January 2019. The protocol was approved by the Microbiology Department, Anbar medical College. All patients provided informed consent for participation in the study and for biopsy samples taken from the tumors and tonsillectomy specimens.

Molecular study of EBV

Patients and Methods:

Sixty-seven paraffin-embedded tissues of NPC cases and 134 normal noncancerous nasopharyngeal biopsy samples and tonsillectomy specimens from patients with chronic hypertrophic tonsillitis as controls were enrolled in the study that was conducted between 3 January 2012 and 1 January 2019 to investigate the presence of LMP-1 gene. Paraffin-embedded tissues of NPC cases were selected from the archives of private pathology laboratories in Anbar province and cases were classified according to the WHO classification [10]. Archived slides were reviewed by two pathologists for confirmation of diagnosis and tissue adequacy for extracting of DNA. Controls were matched on gender, residence and year of birth. The molecular analyses were carried out at private laboratories in Baghdad city.

Tissue Processing, PCR Amplification and Gel Electrophoresis

The tissue samples were purified from archival paraffin embedded tissue blocks as described previously [11]. DNA of EBV was extracted from both cases and controls using the same procedure described previously [12] and PCR amplification for detection of EBV-LMP1 gene were processed through using oligonucleotide (sense BN1, antisense BN2) as follow (sense BN1: 58-AGC GAC TCT GCT GGA AAT GAT-38 or antisense BN2: 58-TGA TTA GCT AAG GCA TTC CCA-38) as described previously [13]. The products were then examined on 1.5% agarose gel electrophoresis in 1× Tris-boric acid–EDTA (TBE) solution and stained with ethidium bromide to verify the presence of 316 bp PCR product.

Statistical Analysis

A matched design and a case-control ratio of 1:2 was selected as the most appropriate strategy in order to maximize the study power. To detect an association with a matched odds ratio of 2.0 at the 5% significance level with 80% power (assuming 20% exposure level among controls), a sample size of 67 cases and 134 controls was required [14].

Epi Info Version 7.02 [15] and SPSS version 24 were used to calculate crude matched odds ratios (OR) with 95% confidence intervals (CI) and two-tailed P-values to estimate the association between various potential risk factors and NPC. Following the univariate analysis, SPSS were used to calculate adjusted odds ratios by conditional binary logistic regression of risk factors with a P-value < or = 0.25.

Results

During the study period, 67 NPC cases were collected and 134 normal lymphatic tissues registered as possible controls. From the total number of controls registered, 134 were included in the study,67cases matched to 2 controls. The demographic characteristics of the cases and controls are shown in Table 1. More males than females were enrolled in the study, with the majority of subject's in age group 19 – 38 (40.3%) and 58 years old and over (49.3%). Regarding residence, rural patients more than urban.

Table 1: Demographic characteristics of subjects

Character	**NPC Cases N= 67 (%)**	**Healthy Control N= 134 (%)**	**P. Value**
Gender Male Female	50 (74.63%)	100 (74.63%)	1.000
	17 (25.37 %)	52 (25.37)	
Residence Rural Urban	42(62.69 %)	84 (62.69 %)	1.000
	25 (37.31 %)	50 (37.31 %)	
Age Group			
<= 18	1(1.5%)	2 (1.5%)	1.000
19 - 38	27 (40.3%)	54 (40.3%)	
39 - 57	6 (9.0%)	12 (9.0%)	
58+	33 (49.3%)	66 (49.3%)	

Detection of EBV LAMP1

EBV were Detected in NPC cases and healthy cases on Agarose gel electrophoresis stained with Rad safe as shown on Fig.1

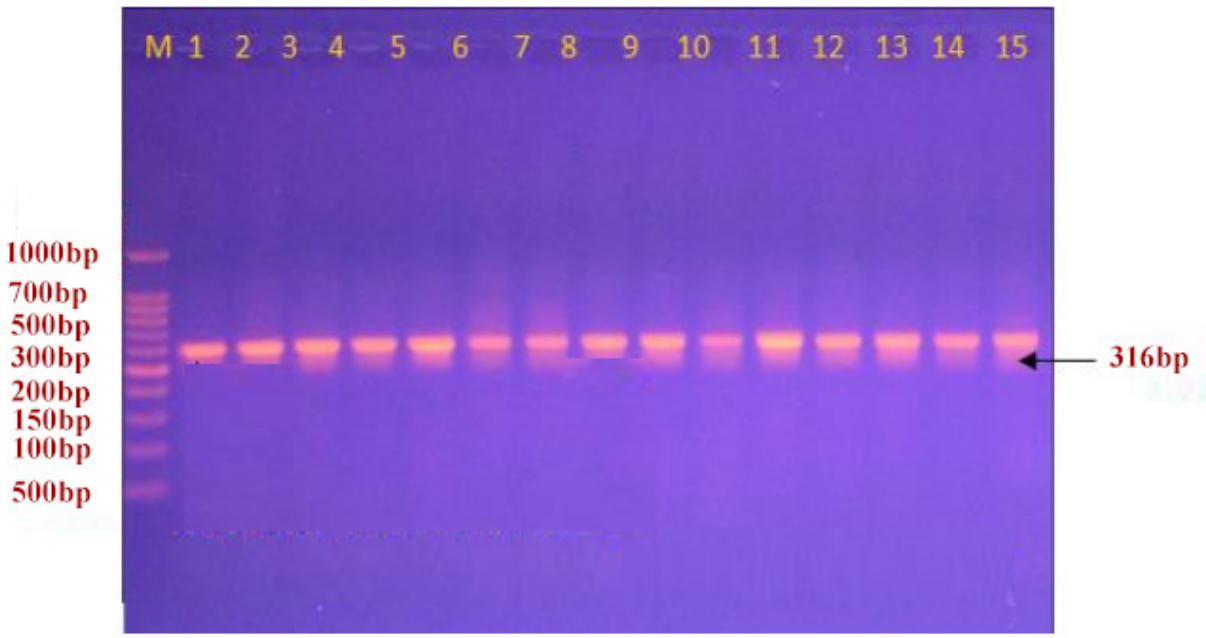


Fig. 1: Detection of EBV in NPC cases and healthy cases on agarose gel electrophoresis stained with Rad safe. Lane M shows a molecular size marker. Lanes 1-15 shows the positive bands of EBV-wt-LMP1 with product size 316 bp.

Univariate analysis of risk factors

Potential risk factors for NPC (i.e. those with a matched odds ratio > 1) are shown in Table 2. Infection with EBV (OR 10.631, CI 3.654-30.930), Herbal Medicine (OR 20.7955, CI 9.531-45.457), history of chronic Rhinitis (OR 3.792, CI 1.069-13.447), Family history of NPC (OR 74.233, CI 9.752-565.051) and Exposed to formaldehyde (OR 2.375, CI 1.303-4.330), were highly and moderate risky and significantly associated with NPC.

Cigarette Smoking (OR 1.309, CI 0.727-2.356) with a low risk of illness, but this was not statistically significant.

In regard to Alcohol drinking (OR 0.715, CI 0.381-1.342), High Background Radiation Areas (OR 0.662, CI 0.068-6.484), Tea consumption (OR 0.557, CI 0.307-1.010) and Dust and/or exhaust exposure/domestic fumes intake (OR 1.031, CI 0.569-1.870) were non significantly associated with NPC

Insufficient data were obtained on Cannabis intake, Salted fish consumption and history of chronic respiratory tract conditions that may be assumed or potential risk factor for NPC, and as such, no conclusions could be made about these exposures.

Table 2: Univariate analysis of selected risk factors for NPC (matched OR > 1)

Risk Factors	NPC Cases N= 67))	Healthy Control N= 134))	Matched Odds Ratio	95% CI	P. Value
EBV Positivity					
Positive	63 (94.03 %)	80 (59.70 %)	10.631	3.654-30.930	0.000
Negative	4 (5.97 %)	54 (40.30 %)			
Herbal Medicine					
Yes	45 (67.16 %)	12 (9.00 %)	20.7955	9.531-45.457	0.000
No	22 (32.84 %)	122 (91.00 %)			
History of chronic Rhinitis					
Yes	7 (10.45 %)	4 (2.99 %)	3.792	1.069-13.447	0.044
No	60 (89.55 %)	130 (97.01 %)			
Family history of NPC					
Yes	24 (35.82 %)	1 (0.75 %)	74.233	9.752-565.051	0.000
No	43 (64.18 %)	133(99.25 %)			
Exposed to formaldehyde					
Yes	36 (53.73 %)	44 (32.84 %)	2.375	1.303-4.330	0.006
No	31 (46.27 %)	90 (67.16 %)			
Cigarette Smoking					
Smoker	35 (52.24 %)	61 (45.52 %)	1.309	0.727-2.356	0.347
Nonsmoker	32 (47.76 %)	73 (54.48 %)			
Alcohol drinking					
Yes	20 (29.85 %)	50 (37.31 %)	0.715	0.381-1.342	0.347
No	47 (70.15 %)	84(62.69 %)			
High Background Radiation Areas					
Yes	1 (1.49 %)	3 (2.24 %)	0.662	0.068-6.484	1.000
No	66 (98.51 %)	131(97.76 %)			
Tea consumption					
Yes	34 (50.75 %)	47(35.07 %)	0.557	0.307-1.010	0.067
No	33(49.25 %)	87(64.93 %)			
Dust and/or exhaust exposure/domestic fumes intake					
Yes	28 (41.79 %)	55 (41.04 %)	1.031	0.569-1.870	1.000
No	39 (58.21 %)	79 (58.96 %)			

Multivariate analysis of risk factors

Of the ten risk factors included in the conditional binary logistic regression model, a significant independent association with illness was found for the following three risk factors: exposure to EBV (adjusted OR 7.852), Herbal Medicine using (adjusted OR 19.051), and Family History NPC (adjusted OR 63.717) (Table 3).

Table 3: Multivariate analysis of selected risk factors for NPC

Risk factor	Adjusted odds ratio	95% CI	P-value
EBV Positivity	7.852	2.224-27.720	0.001
Herbal Medicine using	19.051	7.568-47.953	000
Family History NPC	63.717	6.678-607.963	000

Discussion

The results obtained from our study suggest a strong effect of exposure to EBV, herbal medicine using and family history of NPC on NPC risk, Exposure to formaldehyde and the history of chronic rhinitis were also related to NPC risk, whereas the exposure to high background radiation, cigarette smoking, alcohol drinking, tea consumption and dust and/or exhaust exposure/domestic fumes intake in Anbar province were not related to NPC risk even after adjusting for major risk factors of NPC. Epidemiological study have combined three suspected risk factors (EBV, herbal medicine using and Family History NPC) into a simultaneous analysis factors and study their possible synergetic effects, and reports of relatives with NPC using conditional multivariate logistic regression.

Therefore, it is strongly suspected that NPC risk is affected by cofactor(s) in addition to EBV infection like herbal medicine using and family history NPC were strong risk factors of NPC. This finding is consistent with that of Sriamporn et al.(1992) [16] who showed elevated VCA/IgA and neutralizing antibodies against EBV DNAse with a 20-fold increase in NPC risk for subjects seropositive for VCA/IgA antibodies, and a 30-fold increase for those seropositive for both biomarkers.

Our study agreed with two studies from Taiwan that showed high prevalence of the LMP1. DNA (94.7–100%) in swab samples from NPC patients but not from control groups and other study that demonstrated that NPC is well specimen that can be demonstrate the transforming ability of EBV latent membrane protein which is expressed in approximately 65% of NPC tumors [5, 18, 19], So the plasma EBV-DNA level might be a sensitive and reliable biomarker for the diagnosis of NPC at a molecular level/ clinical practice [20, 21] as EBV is a major etiologic factor for (NPC), and it is detected in tumor cells of virtually all NPC cases [22].

The results from Tables 2 suggest a synergetic effect between herbal medicine using and EBV. These results are in agreement with those obtained by West et al [23] that confirms a 2- to 4-fold excess risk of NPC in association with use of traditional herbal medicines through several case control studies. In contrast to other findings, however, no evidence of herbal medicine using links to NPC was detected [1]. Herbal medicine using might be a strong risk factor of NPC through activation EBV in latent EBV infected cell or through a direct promoting effect on EBV-transformed cells [24] .

Furthermore Family History NPC (indicated as a history of first degree relatives with NPC) was risky for NPC as approved by Busson et al, (2004) [5] who found there is evident that genetic factors might be of importance for the etiology of NPC. The mechanism of familial clustering is not understood and it may reflect genetic factors, shared environmental factors, or both [9]. In accordance with the present results, previous studies have demonstrated that a strong association between our estimates of formaldehydeexposure and NPC [9].

The present study identifies cigarette smoking, Alcohol drinking, Tea consumption and dust and/or exhaust exposure/domestic fumes intake are statistically non-significant risk factors for NPC after adjusting these identified risk factors. These findings are in contrary to some studies that suggested these factors are risk factor for NPC. However, other studies which have suggested

that the relationship of NPC to these risk factors are less clear and inconsistent [1, 7-9].

To our knowledge, this study is the first study in Anbar province that illustrates the association between some risk factors including EBV and NPC development, hoping that a better understanding of the etiologic interactions between viral and environmental factors in the pathogenesis of NPC.

Conclusion

Combination of family history of NPC, EBV exposure and herbal medicine was a strong risk factor for NPC.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Alotaibi AD, Ahmed HG, Elasbali AM. Nasopharyngeal cancer in Saudi Arabia: Epidemiology and possible risk factors. J Oncol Sci. 2019.
2. Sze H, Blanchard P, Ng WT, Pignon J-P, Lee AWM. Chemotherapy for nasopharyngeal carcinoma–current recommendation and controversies. Hematol Clin. 2015;29(6):1107–22.
3. Ferlay J, Colombet M, Soerjomataram I, Dyba T, Randi G, Bettio M, et al. Cancer incidence and mortality patterns in Europe: Estimates for 40 countries and 25 major cancers in 2018. Eur J Cancer. 2018;103:356–87.
4. Yip TTC, Ngan RKC, Fong AHW, Law SCK. Application of circulating plasma/serum EBV DNA in the clinical management of nasopharyngeal carcinoma. Oral Oncol. 2014;50(6):527–38.
5. Busson P, Keryer C, Ooka T, Corbex M. EBV-associated nasopharyngeal carcinomas: from epidemiology to virus-targeting strategies. Trends Microbiol. 2004;12(8):356–60.
6. Ayadi W, Khabir A, Hadhri-Guiga B, Fki L, Toumi N, Siala W, et al. North African and Southeast Asian nasopharyngeal carcinomas: between the resemblance and the dissemblance. Bull Cancer. 2010;97(4):475–82.
7. Sabarimurugan S, Kumarasamy C, Baxi S, Devi A, Jayaraj R. Systematic review and meta-analysis of prognostic microRNA biomarkers for survival outcome in nasopharyngeal carcinoma. PLoS One. 2019;14(2):e0209760.
8. Feng BJ, Khyatti M, Ben-Ayoub W, Dahmoul S, Ayad M, Maachi F, et al. Cannabis, tobacco and domestic fumes intake are associated with nasopharyngeal carcinoma in North Africa. Br J Cancer. 2009;101(7):1207.
9. Yang XR, Diehl S, Pfeiffer R, Chen C-J, Hsu W-L, Dosemeci M, et al. Evaluation of risk factors for nasopharyngeal carcinoma in high-risk nasopharyngeal carcinoma families in Taiwan. Cancer Epidemiol Prev Biomarkers. 2005;14(4):900–5.
10. Hamilton SR, Aaltonen LA. WHO classification of tumours. Pathology and genetics of tumours of the digestive system. Geneva World Heal Organ. 2000;
11. Chen BF, Clejan S. Rapid preparation of tissue DNA from paraffin-embedded blocks and analysis by polymerase chain reaction. J Histochem Cytochem. 1993;41(5):765–8.
12. Tseng CK, Tsang NM, Kao SC, Chen SY, Chen YP. A quick method to extract DNA from paraffin-embedded tissues. Chang yi xue za zhi. 1998;21(1):63–6.
13. Lin S, Tsang N, Kao S, Hsieh Y, Chen Y, Tsai C, et al. Presence of Epstein-Barr virus latent membrane protein 1 gene in the nasopharyngeal swabs from patients with nasopharyngeal carcinoma. Head Neck J Sci Spec Head Neck. 2001;23(3):194–200.
14. Schlesselman JJ. Case-control studies: design, conduct, analysis. Vol. 2. Oxford University Press; 1982.
15. Dean AG, Sullivan KM, Zubieta J, Delhumeau C. Epi Info 2000: a database, and statistics program for public health professionals using Windows® 95, 98, NT, and 2000 computers. 2000;
16. Sriamporn S, Vatanasapt V, Pisani P, Yongchaiyudha S, Rungpitarangsri V. Environmental risk factors for nasopharyngeal carcinoma: a case-control study in northeastern Thailand. Cancer Epidemiol Prev Biomarkers. 1992;1(5):345–8.

17. Tiwawech D, Srivatanakul P, Karalak A, Ishida T. Association between EBNA2 and LMP1 subtypes of Epstein-Barr virus and nasopharyngeal carcinoma in Thais. J Clin Virol. 2008;42(1):1–6.
18. Young LS, Dawson CW. Epstein-Barr virus and nasopharyngeal carcinoma. Chin J Cancer. 2014;33(12):581.
19. Endo K, Kondo S, Shackleford J, Horikawa T, Kitagawa N, Yoshizaki T, et al. Phosphorylated ezrin is associated with EBV latent membrane protein 1 in nasopharyngeal carcinoma and induces cell migration. Oncogene. 2009;28(14):1725.
20. Han B-L, Xu X-Y, Zhang C-Z, Wu J-J, Han C-F, Wang H, et al. Systematic review on Epstein-Barr virus (EBV) DNA in diagnosis of nasopharyngeal carcinoma in Asian populations. Asian Pacific J Cancer Prev. 2012;13(6):2577–81.
21. Fan H, Nicholls J, Chua D, Chan KH, Sham J, Lee S, et al. Laboratory markers of tumor burden in nasopharyngeal carcinoma: a comparison of viral load and serologic tests for Epstein-Barr virus. Int J cancer. 2004;112(6):1036–41.
22. Chen Y, Xu Y, Zhao W, Xiao X, Zhou X, Lin L, et al. Lack of association between cigarette smoking and Epstein Barr virus reactivation in the nasopharynx in people with elevated EBV IgA antibody titres. BMC Cancer. 2018;18(1):190.
23. West S, Hildesheim A, Dosemeci M. Non-viral risk factors for nasopharyngeal carcinoma in the Philippines: results from a case-control study. Int J cancer. 1993;55(5):722–7.

Molecular Detection of Toxogenic Cyanobacteria Isolated from Tigris River in Baghdad City –Iraq

Zainulabdeen H. A. AL-Khafaji[1], Ahmed S.Dwaish[2]

[1]Department of Biology ,College of Education, University of Mosul.(Msc.Assist.lecture) [2]Department of Biology , College of Science, Mustansiriyah University. (PhD.Assist.Professor)

Abstract

Algae and their contamination are being increasingly reported worldwide that cause a serious hazard to environmental and human health. Cyanotoxin was the most algal toxin reported to be produce by several orders of cyanobacteria. In 2017 cyanobacteria were isolated from fresh water of Tigris River and identified by light compound microscope as well as conventional PCR. Five isolates of cyanobacteria which successfully amplified a gene fragment from the phycocyanin shared by all cyanobacteria and only four isolates successfully amplified a gene fragment from the myc E belonged to microcystin. Our results concluded that PCR assay can be used for early detection of microcystin producing algae in fresh water that useful to stations responsible for the preparation of drinking water.

Keywords: *Algae, Cyanotoxin, phycocyanin and microcystin.*

Introduction

Cyanobacteria, cyanophytes or blue green algae are widely distributed in natural environments and are considered a major component of microbial populations in terrestrial and aquatic habitats worldwide, Harmful algal blooms have been identified in fresh water, Estuarine, and marine system. In fresh water some cyanobacterial may produce dermal toxins, neurotoxins and hepatotoxins which including nodularins and microcystins [(1).]

They are also an interesting functional food source [(2)] . These microbes have also been reported to be rich sources of healthy nutrients such as proteins, carbohydrates, vitamins, minerals amino acids, and fatty acids.

Among all the cyanotoxins, microcystins are the most frequently studied because of their wide distribution and high toxicity. Up to now, more than 80 different structural variants have been identified, among which microcystin-LR is the most common and potent variant, followed by microcystin-RR and microcystin-YR [(3).]

Monitoring systems are needed to prevent water users from these toxins. Good methods, such as ELISA and high performance liquid chromatography have been recorded for most cyanotoxins, but they extremely use laborious sample preparation protocols as well as priced machinery and purified toxin standards that are often difficult to obtain. Nevertheless, molecular detection techniques such as conventional PCR, quantitative real-time PCR and microarrays/DNA chips that are rapid, extremely sensitive and specific for detecting toxic cyanobacteria in water supplies [(4; 5).]

The delivery of phycocyanin in the cyanobacterial makes the study of phycocyanin genes good idea for the classification of cyanobacteria [(6).] Phycocyanin operon contains genes coding for two bilin subunits and three linker polypeptides. The intergenic spacer (IGS) between the two bilin subunit genes, designated as b (*cpcB*) and a (*cpcA*) showed variations in their sequences which are useful of differentiating genotypes below the generic level make it capable for the identification of cyanobacteria via PCR [(7).]

Address for Correspondence:
Dr. Ahmed S.Dwaish
Biology Department College of Science Mustansiryih University, Baghdad – Iraq
E-mail: ahmedsahi33@gmail.com
E-mail: ahmedsahi@uomustansiriyah.edu.iq

Conventional PCR could be usful to detect microcystin producers and several primers are available [8]. *mcy*E primers were successfully used to get PCR product from all known microcystin and nodularin producers [9].

Baghdad city have two main sources of drinking water for Iraq, Tigris River is the most important once in serving population approximately seven million people settled in this city, this river usually affected by industrial eutrification as well as the sewage effluent and agricultural which provide enhancement of cyanobacteria growth and potential mycrocystin production. Therefore, this study was aimed to use Molecular PCR technique for identification of cyanobacteria and mycrocystin producing isolates from Tigris River for early detection of toxic species that could be useful to companies responsible for the provider of drinking water to this city.

Material and Method

Water samples were collected from the higher superficial layer of Tigris River from a depth of 20-30 cm monthly in April 2017 by using 20µ mesh net. Sampling site located in civil region in AL-Greaat region near AL-Greaat bridge , located on longitude 44°20'37.52"E and latitude 33°25'3.49"N.Samples were transported to the laboratory on ice. 10 ml of water sample was added to chu-10 culture medium and incubated at 28ºC for two weeks with continuous illumination of 50 µE/m²/s. one ml of growth inoculated on agar plates containing BG-11 and incubated in the same condition for one week to isolate unialgal [10]. Microscopic examination were performed to ensure the culture were unialgal.

Extraction of DNA from chlorophyta and cyanbacterial isolates

Genomic DNA was extracted from the chlorophyta isolates for specificity test using CTAB method [11]. While the Genomic DNA was extracted from the cyanobacterial isolates using the genomic DNA mini Kit(plant)

Polymerase chain reaction test

Polymerase chain reaction was performed with two sets of primers. PCβF (GGCTGCTTGTTTACGCGACA) and PCαR (CCAGTACCACCAGCA ACTAA) [12] to amplify *cpcB*-IGC-*cpcA* region in phycocyanin operon while the HEPF (TTTGGGGTTAACTTTTTTGGG CATAGTC) and HEPR (AATTCTTGAGGCTGTAAATCGGGTTT) [13] used to amplify *mcy*E gene of the microcystin synthetase. PCR protpcols involved an initial denaturation for 2 min at 95ºC; 35 cycles of denaturation for 90 sec at 95ºC, annealing for 30 sec at 52ºC (PCβ-PCα primer set) and for 90 sec at 95ºC(HEP primer set), extension for 1min at 72ºC and final extension for 8 min at 72ºC. 10µl of PCR product was separate in 1.5% agarose gel electrophoresis stained with ethidum bromide and visualized on a UV transilluminator, the size of amplified products were compared with the 100pb DNA ladder to determine the exact size of these products.

Results

Isolation and identification of algae from water samples five isolates of cyanobacteria were obtained from the Tigris River included, *Westellopsis sp , Oscillatoria sp* , *Spirulina sp , Chroococcus sp and Lyngbya sp* . Which belonged to four cyanobacterial orders: Oscillatoriales, Chroococales, Stigonematales and Nostocales as well as one isolate of chlorophyceae included *Cladophora glomereta* (Macro algae) where used as negative control test.

Molecular detection of cyanobacteria by PCR test

The gene fragment of the phycocyanin operon containing the IGS (*cpcBA*-IGC) from cyanobacteria was amplified. A distinct amplicon patterns was produced from all of the DNA extracts with a size of 650 bp when analyzed in gel electrophoresis (Fig. 1), confirming the presence of cyanobacterial DNA from isolates collected from fresh water of Tigris River in Baghdad. While lysates of a green alga *Cladophora glomereta* does not possess pycocyanin operon, gave no PCR product suggested the highly specificity of used primers.

Detection of Microcystin by PCR assay

In this study, conventional PCR used as a tool to identify potentially microcystin producing cyanobacteria possess aminotransferase enzyme. The HEP primers were successfully amplified the 472 bp fragments of *mcy* E gene from all microcystin-producing cyanobacterial isolates except *spirulina sp.* (Fig. 2). The specificity of HEP primers appeared to be highly specific for isolates producing microcystin since there was no DNA amplified from chlorophyta used in this study.

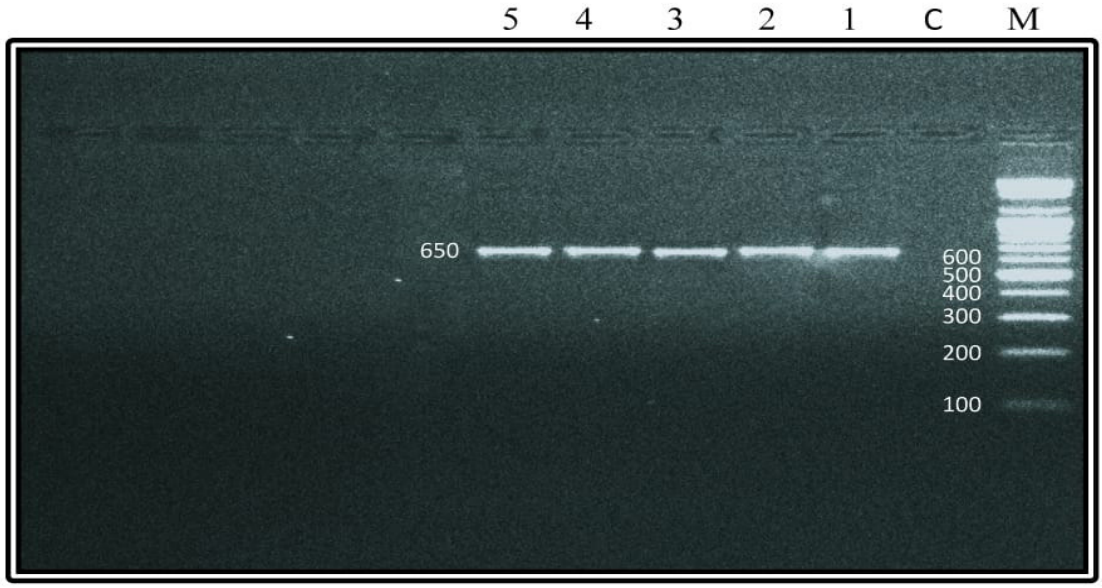


Fig. 1. Gel electrophoresis of amplified *cpcBA-IGC* (650bp) in cyanobacterial isolates. Agarose (1.5%), 5 V/cm for 2 hrs, stained with ethidum bromide and visualized on a UV transilluminator. M. 100 bp DNA ladder. Lane 1-5 . *Westellopsis sp* , *Oscillatoria sp* , *Spirulina sp* , *Chroococcus sp and Lyngbya sp* . Lane C. *Cladophora glomereta*. M.Marker.

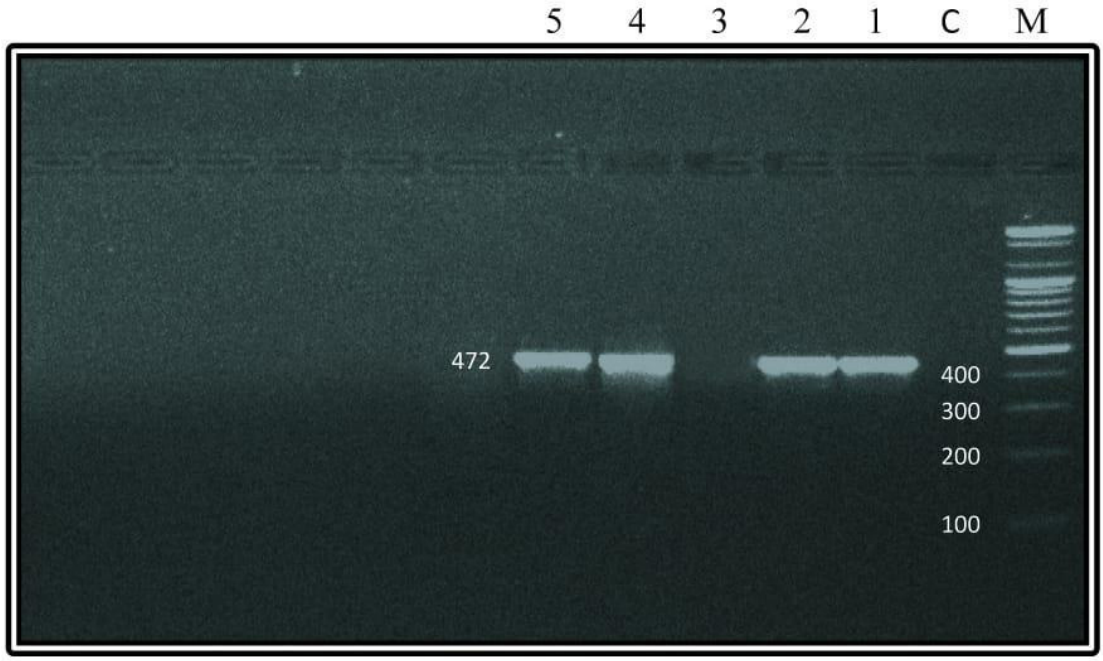


Fig. 2. Gel electrophoresis of amplified *mycE* (472bp) in cyanobacterial isolates. Agarose (1.5%), 5 V/cm for 2 hrs, stained with ethidum bromide and visualized on a UV transilluminator. M. 100 bp DNA ladder. Lane 1-5 . Westellopsis sp , Oscillatoria sp , Spirulina sp , Chroococcus sp and Lyngbya sp .Lane 6. Cladophora glomereta. 7.Negative control.

Discussion

The microscopic results revealed that all species of cyanobacteria that isolated from Tigris River related to toxic dominant genera which produce microcystins except *spirulina platent*. This might be related to the capability of these species to highly competition to remain dominant utilizing all environmental conditions such as high temperature, optical density and abundance of nutrients , all these factors allowed to form the blooming and can increase microcystin production rates (14).

Morphological identification is time consuming and it requires high expertise. In fact, morphological features used for the identification of species such as

colonial form, mucilage patterns and cell arrangement in the colony is frequently variable and dependent on the environment [15]. Furthermore, the co-occurrence of toxin producing and non-producing cells that are morphologically indistinguishable [16; 17]. Therefore, the development of a molecular method for the identification of cyanobacteria is essential for the rapid and accurate analysis members of cyanobacterial population [6]. Several investigators used PCβ-PCα primer set for cyanobacterial detection and showed the same results revealed in this study [12; 18]. Except in [6] study, he was reported that phycocyanin gene fragments from *Nostoc commune* and *Nostocpunctiforme* were unable to be amplified using these primers while strains of all of the cyanobacterial genera were successfully amplified. In recent research, found that *Nostoc punctiforme* had short sequence and incomplete of *cpcBA*-IGC region resulting in high variability in these genes and cause heterogeneity of genus *Nostoc*[19].

The detection of cyclic peptide hepatotoxin genes by using HEPF and HEPR primers was developed to identify potentially microcystin or nodularin-producing cyanobacterial blooms that possess the aminotransferase domain of either *mcy E* or *nda F*, involved in the production of microcystin or nodularin from four order of cyanobacteria included *Oscillatoriales, Chroococales, Stigonematales* and *Nostocales* [13].

The aminotransferase domain was chosen as the target sequence because of its essential function in the synthesis of all microcystins as well as nodularins that catalyzes the addition of D- glutamate to Adda, an essential step in the synthesis of both microcystin and nodularin [20]. Thus, can use these described primers to amplify a 472 bp PCR product from the aminotransferase domains of all tested hepatotoxic species and bloom samples. In adittion, these primers can be used for distinguished between toxic and non-toxic populations of cyanobacteria that coexist simultaneously in a single ecosystem and are indistinguishable by microscopy [13].

Tigris River usually affected by agricultural and industrial eutrification as well as the sewage effluent, high turbidity, river discharge or by agricultural runoff which provide protected mesocosms of cyanobacteria growth and potential microcystins production. The results by [21] and [22], suggested that eutrophication increased the co-occurrence of potentially microcystins producing cyanobacterial genera, raising the risk of toxic-bloom formation.

References

1. Nonneman, D. and Zimba, P.V. A PCR-based test to assess the potential for microcystin occurrence in channel catfish production ponds. J.phycol.2002., 38: 230-233.
2. Pandey U. and Pandey J., Enhanced production of biomass, pigments and antioxidant capacity of a nutritionally important cyanobacterium Nostochopsis lobatus, Bioresour. Technol., 2008; 99: 4520-4523.
3. Chen, L., Zhang, X., Zhou,W., Qiao,Q., Liang, H., Li, G., Wang, J. and Cai, F.The interactive effects of cytoskeleton disruption and mitochondria dysfunction lead to reproductive toxicity induced by microcystin-LR. PLOS ONE. 2013., 8(1): e53949.
4. Pearson, L.A. and Neilan, B.A. The molecular genetics of cyanobacteria toxicity as a basis for monitoring water quality and public health risk. Current Opinion in Biotechnology.2008., 19:281-288.
5. Sivonen, K. Emerging high throughput analyses of cyanobacterial toxins and toxic cyanobacteria. Adv. Exp. Med. Biol.2008 ., 619: 539-57.
6. Neilan, B.A., Jacobs, D. and Goodmann A. Genetic diversity and phylogeny of toxic cyanobacteria determined by DNA polymorphisms within the pycocyanin locus. Appl. Environ. Microbiol.1995., 61(11): 3875-3883.
7. Wu, Z.,Shi, J., Lin, S. and Li, L. Unraveling molecular diversity and phylogeny of *Aphanizomenon*(Nostocales, Cyanobacteria) strains isolated from China. J.phycol. 2010., 46(5):1048-1058.
8. Welker, M., Christiansen, G. and Van Döhren, H. Diversity of coexisting *Planktothrix* (cyanobacteria) chemotypesdedused by mass spectral analysis of microsystins and other oligopeptides. Arch. Microbiol.2004., 182(4):288-98.
9. Rantala, A., Wacklin, P.R., Lyra, C., Lepisto, L., Rintala, J., Boczek, J.M. and Sivonen, K. Detection of microcystin-producing cyanobacteria in Finnish lakes with genes-specific microcystinsynthetase gene E (mcyE) PCR and associations with environmental factors. Appl. Environ. Microbiol .2006., 72(9): 6101-6110.
10. Stein, J.R. Handbook of phycological methods: Culture methods and growth measurements.

Cambridge University Press. Cambridge.1973.

11. Doyle, J.J and Doly, J.L. A rapid total DNA preparation procedure for fresh plant tissue. Focus.1990; 12:13-15.
12. Saker, M.L., Vale, M., Kramer, D. and Vasconcelos, V.M. Molecular techniques for the early warning of toxic cyanobacteria blooms in freshwater lakes and rivers. Appl. Microbiol. Biotechnol.2007., 75:441-449.
13. Jungblut, A.D. and Neilan, B.A. Molecular identification and evolution of the cyclic peptide hepatotoxins, microcystin and nodularin, synthetase genes in three orders of cyanobacteria. Archives of Microbiology. 2006.,185: 107-114.
14. Kearns, K.D and Hunter, M.D. Toxin-producing *Anabaenaflos-aquae* induces settling of *Chlamydomaonasreinhardttii* a Competing Motile Algae. Microb. Ecol.2001., 42(1): 80-86.
15. Otsuka, S., Suda, R. H. Li, Matsumoto, S. and Watanabe, M. M. Morphological variability of colonies of *Microcystis*morphospecies in culture; J. Gen. Appl. Microbiol.2000., 46: 39-50.
16. Janse I, Kardinaal, WE, Meima, M, Fastner, J, Visser, PM, Zwart, G.Toxic and nontoxic *Microcystis* colonies in natural populations can be differentiated on the basis of rRNA gene internal transcribed spacer diversity. Appl. Environ. Microbiol.2005., 70(7):3979-87.
17. Dittmann, E. and Wiegand, C. Cyanobacterial toxins occurrence biosynthesis and impact on human affairs. Mol. Nutr. Food Res .2006., 50:7–17.
18. Baron-Sola, A., Ouahid, Y. and delCampo, F.F. Detection of potentially producing cylindrospermopsin and microcystin strains in mixed populations of cyanobacteria by simultaneous amplification of cylindrospermopsin and microcystin gene regions. Ecotoxicology and Environmental Safety. 2012., 75:102–108.
19. Teneva, I., Stoyanov, P., Mladenov, R. and Dzhambazov, B. Molecular and phylogenetic characterization of two species of the genus *Nostoc*(cyanobacteria) based on the *cpcB-IGS-cpcA*locus of the phycocyanin operon. J. BioSci. Biotech .2012., 1(1): 9-19.
20. Al-Tebrineh, J., Gehringer, M.M., AKcaelon, R. and Neilan, B.A. A new quantative PCR assay for the detection of hepatotoxigenic cyanobacteria. Toxicon.2011; 57:546-554.
21. Rantala, A., Fewer, D.P., Hisbergues, M., Rouhiainnen, L., Vaitomaa, J., Börner, T. and Sivonen, K. Phylogenetic evidence for the early evolution of microcystin synthesis. Proceedings of the National Academy of Sciences of the United States of America.2004; 101(2): 568-573.
22. Bittencourt-Oliveira, M.C., Oliveira, M.C. and Pinto, E. Diversity of microcystin-producing genotype in brazilian strains of microcysis (cyanobacteria). Braz.J.Bio.2011.,71(1):209-216.

Flame Atomic Absorption Spectrophotometry Analysis of Heavy Metals in Some Food Additives Available in Baghdad Markets, Iraq

Wasan Abdulwahid Jasim[1], Jassim Dawood Salman[2], Jasim Mohammed Shamar Jamur[3]

[1]Assistant Lecturer, [2]Assistant Professor, [3]Dr. Lecturer, Department of Chemistry, College of Education for Pure Science (Ibn Al- Haitham), University of Baghdad, Iraq

Abstract

Flame atomic absorption spectrophotometer (FAAS) was used in this study to determine the concentrations of heavy metals such as Ca, Fe, Mn, Cd, Co, Cr, Ni, Cu, Pb and Zn in some food additives of Iraq. The order of metal contents in food additives was found to be Ca > Mn > Fe > Cu > Zn > Pb > Cr > Ni > Co > Cd. The concentration level of each metal was compared with that recommended by food agriculture organisation (FAO) and world health organisation (WHO). Calibration curves were linear for all standard solutions of heavy metals in the range starting from 0.02-0.4 mg/kg for Cd to 11-100 mg/kg for Ca. The correlation coefficients values (R^2) of calibrations were investigated and ranged from 0.9971 for Cr to 0.9999 for Ca. The limit of detection (LOD) and limit of quantification (LOQ) were found to be in highest value for Ca (1.6569 mg/kg and 5.5232 mg/kg), while they were found to be in lowest values for Cd (0.0150 mg/kg and 0.0499 mg/kg).

Keywords: *Heavy metals, Food additives, Flame atomic absorption spectrophotometer*

Introduction

Several elements are important for a wide range of biological processes due to their conjugation with proteins to form metalloproteins which are an important component in the enzymatic systems[1,2]. Despite the importance of some metals including Cr, Al and Ni for plants, they can be toxic with high concentrations[3]. Furthermore, some heavy metals such as Pb and As in food are a major health problem due to their carcinogenic effects[4]. In addition, the presence of metals in soils during wastewater are environmental problem, and the main cause of the agricultural pollution[5].

The last three decades have seen increasing the production of spices about 3.5% per year, and play a key role in the history of civilisation around the world[6,7]. Although spices are essential, there is increasing concern that some of them are being disadvantaged. This attributed to the accumulation of toxic trace elements[8]. In addition, the spices may be contaminated with trace and heavy metals, and this could led to health problems[9]. Therefore, researches have shown an increased interest in examine the effect of metals on air, water and food and their impact on human consumption. Concentrations of trace and heavy metals in spices are an important components in the healthcare systems due to their medicinal effective on human health[8]. Some metals such as lead (Pb), arsenic (As), nickel (Ni) and cadmium (Cd) can be extremely harmful to human beings even in trace levels[10]. However, spices are one of the most widely used groups of antibacterial, antioxidant and anti-diabetic agents[11].

A number of studies have been made to investigate the concentration of metals in spices[12]. On the other hand, information about the efficacy of heavy metals and safety of spices are limited[13]. The heavy metals in food need to be monitored in order to protect human and animals from the hazards of these metal ions; WHO has determined the maximum level for each toxic element in foodstuffs[8]. Several analytical methods have been used to determine the metal concentrations in spice samples of

Corresponding author:
Jasim jamur,
E-mail: jasimphduk@gmail.com

different countries. Krejpcio and co-workers have shown the quantification analysis of some heavy metals such as Cd, Pb, Cu and Zn in popular spices used in Polish market using atomic absorption spectrophotometer (AAS)[11]. In the study by Nkansah and Amoako, AAS has been successfully used to monitor the concentration levels of heavy metals including Ni, Fe, Cu, Co, Pb and Zn in many different types of spices in Ghana[9]. The quantification analysis of some toxic metals such as As, Pb, Cd and Ni in a number of common spices in Pakistan using AAS method were carried out by Baig *et al*[8]. In a study conducted by Karadas and Kara, it was shown that geometric methods such as principal component analysis (PCA) and cluster analysis (CA) can be used to analyse the trace metal concentrations such as Co, Mn, Fe, Mg, Cr, Cu, Ca, Cd, Ba, As and Sr in some spices in Turkey[15].

The experimental work presented here provides the levels of heavy metals in some food additive available in the local markets of Baghdad, Iraq. This study therefore set out to assess the effect of some heavy metals such as Ca, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in some popular food additive in Iraq such as qayima, dolma, kubba, seven, pizza, biryani, judur, chicken, kubsa, burger and sumac using FAAS technique. Both qualitative and quantitative methods were used in this investigation.

Materials and Method

Apparatus

Flam atomic absorption spectroscopy (FAAS) model AA-7000 Shimadzu was used as instrumental detection system using hollow cathode lamps. Absorbance measurements of blank (solvent) were recorded using deuterium lamp. Air/ acetylene burner head was used as a carrier gas for all samples. The wavelength used for each metal was: Ca (422.7 nm), Fe (248.3 nm), Mn (279.5 nm), Cd (228.8 nm), Co (240.7 nm), Cr (357.9 nm), Ni (232 nm), Cu (324.8 nm), Pb (283.3 nm) and Zn (213.9 nm).

Chemicals

All chemicals were used without any further purification and all solutions were prepared by deionised water. Nitric acid (65 %) was supplied by Riedel-de Haen. Standard solution of each metal that used for the calibration curve was prepared by stock solution (1000 mg/ L) supplied by Aldrich. Deionised water was provided by Daihan Labtech CO., LTD (Model WD-2008F) with a volts of 220V 50 Hz.

Preparation of samples

Eleven samples of food additives such as qayima, dolma, kubba, seven, pizza, biryani, judur, chicken, kubsa, burger and sumac that used in the study were collected by the local markets of Baghdad, Iraq. One gram of each sample was weighed and digested by mixing with 2 ml of concentrated nitric acid and heated for 1 hour. Then, the produced samples were cooled at room temperature. After digestion, the samples were filtered and diluted to 25 ml with deionised water.

Analysis of sample

All prepared sample solutions were analysed by FAAS method to determine the concentrations of heavy metals such as Ca, Fe, Mn, Cd, Co, Cr, Ni, Cu, Pb and Zn that used in this study. Blank solution was also measured before the sample analysis using the same conditions.

Quantification

For quantification, calibration curves of five different concentrations of each standard solution were applied. All calibrations curves showed a good linear correlation between the concentrations of standard solutions and absorbance. Some statistical analysis including the standard deviation (S.D.), standard error of the mean (S.E.M.) and correlation of coefficients (R^2) were done in order to check the validation of FAAS method. All R^2 were found to be ≥ 0.997. The limit of detection (LOD) and the limit of quantification (LOQ) were also determined by considering standard deviation to the slope:

STEYX means the standard deviation of the y-value and x-value.

Results and Discussion

FAAS has been used to determine many heavy metals such as Ca, Fe, Mn, Cd, Co, Cr, Ni, Cu, Pb and Zn in some food additives such as qayima, dolma, kubba, seven, pizza, biryani, judur, chicken, kubsa, burger and sumac. The results obtained from the analysis are presented in Table 1. The samples were prepared in the same solvent used for the analysis. The wavelength was set for each element (see the material and method section). It can be seen from the date in Table 1 that the level of Ca in all samples is higher than levels observed

of other metals. Interestingly, the samples of qayima and judur did not detect any value of Cr and Co. The results of the study are in agreement with those obtained by WHO. Therefore, there was no evidence that the levels of heavy metals concentrations in these food additives has an influence on human consumption.

Table 1: Contents of heavy metals in different food additives in mg/kg

Heavy metals Food additives	**Ca**	**Fe**	**Mn**	**Cd**	**Co**	**Cr**	**Ni**	**Cu**	**Pb**	**Zn**
Qayima	45.840	6.657	6.519	0.009	0.018	0.000	0.028	0.291	0.024	1.106
Dolma	48.146	5.785	7.146	0.005	0.018	0.030	0.028	2.148	0.048	1.056
Kubba	40.089	3.133	6.188	0.007	0.016	0.008	0.024	1.442	0.072	0.836
Seven	47.985	6.184	6.362	0.005	0.015	0.015	0.046	3.047	0.167	1.182
Pizza	47.725	5.134	6.559	0.015	0.034	0.060	0.055	0.402	0.127	0.842
Biryani	62.550	5.889	7.611	0.023	0.015	0.067	0.041	0.334	0.270	1.360
Judur	37.324	6.683	4.710	0.012	0.000	0.104	0.009	0.273	0.040	0.928
Chicken	45.555	6.562	5.930	0.006	0.023	0.052	0.047	2.414	0.095	0.970
Kubsa	56.055	6.167	5.888	0.005	0.030	0.074	0.043	1.101	0.048	0.993
Burger	69.058	6.258	4.843	0.008	0.033	0.037	0.110	0.855	0.087	0.575
Sumac	7.896	3.329	1.319	0.016	0.018	0.134	0.129	1.668	0.024	1.031

The results obtained from the Figure 1, it can see that the burger recorded the highest level of Ca (69.058 mg/kg), whereas the lowest level was found in sumac (7.896 mg/kg). From the data in Figure 1, it can see that the study resulted the concentration levels of Fe in the samples and found to be between 3.133 mg/kg in kubba and 6.683 mg/kg in judur. These values are lowest than that of determined by FAO and WHO, 2009 (20 mg/kg). Compared to permissible limit of FAO and WHO, 1984 (2 mg/kg), there was the higher Mn content in all food additives samples (> 4 mg/kg) except sumac, Figure 1.

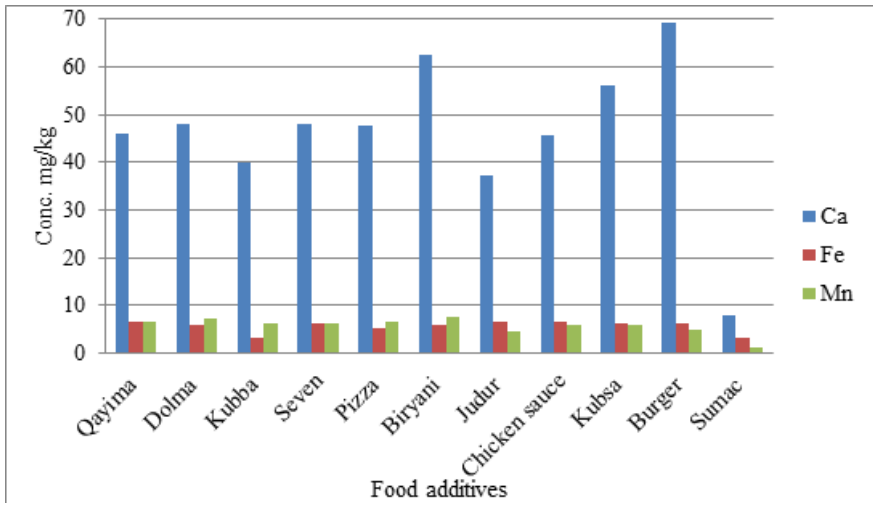


Figure 1:Levels of Ca, Fe and Mn in different food additives

As can be seen from the Figure 2, the concentrations of cadmium in all food additives were lower than that determined by FAO and WHO, 1984 (0.2 mg/kg). They found to be in the range from 0.005 mg/kg in dolma to 0.023 mg/kg in biryani. As shown in Figure 2, the concentrations of "Co" in all samples were under the permissible limit of FAO and WHO, 1984 (0.4 mg/kg). They were in the range of 0.015- 0.034 mg/kg in seven, biryani and pizaa, whereas judur sample did not show any content of Co. Only trace amounts of Cr were detected in the food additives samples, however, FAO/WHO (2009) suggested that the concentration of Cr should be zero in these samples. When Cr was found from 0.008 mg/kg in kubaa to 0.134 mg/kg in sumac, no amount in qayima was detected. The results also shows that the greatest value of Ni was in sumac (0.129 mg/kg), while the lowest value was in judur (0.009 mg/kg). This data can be compared with that determined by FAO and WHO, 1984 (1.63 mg/kg). As shown the maximum obtained values were too far from the permissible limit of FAO and WHO, therefore, the selective food additives in this study appeared to be unaffected by these levels of Ni.

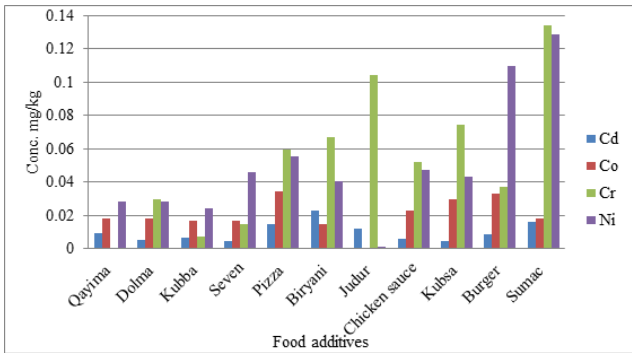


Figure 2: Levels of Cd, Co, Cr and Ni in different food additives

It can be seen from the Figure 3 that the food additives samples reported significantly high concentrations of copper compared to those of some heavy metals such as Cd, Co, Cr, Ni and Pb. It found to be between 0.273 mg/kg in judur and 3.047 mg/kg in seven. However, these observed concentrations were in general under the maximum limit of FAO and WHO, 1984 (3.00 mg/kg). From the data in this Figure, it is apparent that the Pb levels in food additives were higher than those of Cd and Co and lower than other metals. The range of concentrations were found from 0.024 mg/kg in both qayima and sumac to 0.270 mg/kg in biryani. This results indicated there was no evidence that Pb has an influence on human health due to their levels were under the permissible limit of FAO and WHO, 1984 (5.00 mg/kg). The results also shows that the levels of Zn ranged from 0.575 mg/kg in burger to 1.360 mg/kg in biryani. Therefore, they were too far from the maximum level that recommended by FAO and WHO, 2009 (50 mg/kg).

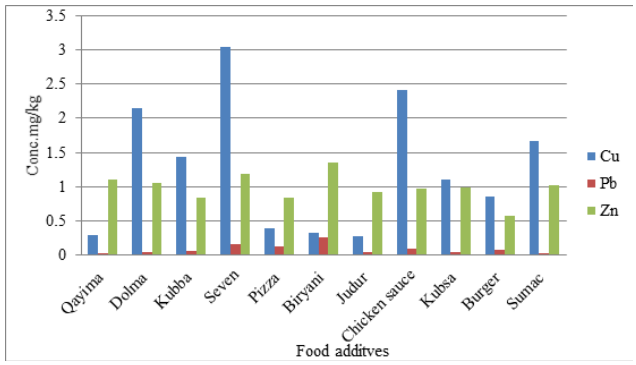


Figure 3: Levels of Cu, Pb and Zn in different food additives

Calibration curves

Correlation analysis of five different concentrations including the blank of each metal were tested to predict the linearity. Calibration ranges of standard solutions are set out in Table 2. From the Figure 4, it can see that all calibrations showed a good linearity over the range of concentrations. The LOD and LOQ of Ca is expected to be higher than others, whereas for Cd they were found to be lower than others . Other values of LOD and LOQ were listed in Table 2. Standard deviation (S.D.) and standard error of the mean (S.E.M.) were calculated by the equations:

$$S = \sqrt{\frac{\sum_{i=1}^{n}(xi - \bar{x})2}{n-1}}$$

$$\text{S.E.M.} = \frac{s}{\sqrt{n}}$$

Where *S* is standard deviation, *n* is number of values in the sample, *xi* is data value. $\bar{x}$ sample mean, S.E.M. is standard error of the mean and *s* is size of the sample. The results of the mean, S.D. and S.E.M. are summarised in Table 2.

Table 2: Data of linear regression, sensitivity and precision of the FAAS method of the FAAS method

Metal	Calibration Range (mg/kg)	Corr. R2	LOD (mg/kg)	LOQ (mg/kg)	S.D.	S.E.M.
Ca	11-100	0.9999	1.6569	5.5232	15.7482	4.7482
Fe	0.5-10	0.9992	0.3813	1.2825	1.2603	0.3800
Mn	0.4-7.0	0.9991	0.2975	0.9959	1.6972	0.5117
Cd	0.02-0.4	0.9993	0.0150	0.0499	0.0057	0.0017
Co	0.4-4.0	0.9991	0.1696	0.5654	0.0096	0.0029
Cr	0.1-2.0	0.9971	0.1505	0.5019	0.0412	0.0124
Ni	0.1-2.0	0.9997	0.0518	0.1727	0.0372	0.0112
Cu	0.3-4.0	0.9987	0.1974	0.6581	0.9595	0.2894
Pb	2.5-10	0.9988	0.4823	1.5951	0.0741	0.0223
Zn	0.1-1.0	0.9997	0.0209	0.0698	0.2038	0.0614

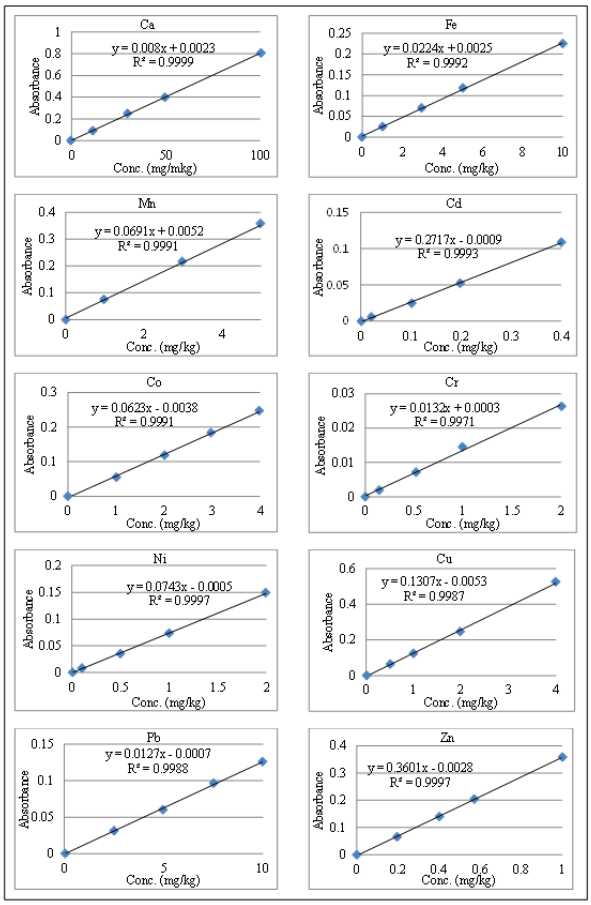


Figure 4. Calibration curves for the standard solutions of heavy metals by FAAS

CONCLUSION

The present study was designed to determine the levels of some heavy metals in different samples of food additives available at local markets in Baghdad, Iraq using FAAS technique. This has shown that the method is simple, fast and sensitive. The obtained concentrations of metals were acceptable with that recommended by FAO and WHO. The study has shown that highest correlation coefficient (R^2) of calibrations was found to be for Ca (0.9999), while the lowest was found to be for Cr (0.9971). The LOD and LOQ for Cd were found to be lower than others (0.0150 mg/kg and 0.0499 mg/kg) followed by Zn (0.0209 mg/kg and 0.0698 mg/kg). This could attributed to that the Cd and Zn are sensitive enough even at low concentrations.

For recommendations, the findings of this study have several important applications for future work. The method can be applied for determination of other metals in different samples of foodstuffs. Further research should also focus on determining the toxic and carcinogenic heavy metals in food additives and how can be able to reduce their effects by reducing their amounts.

Acknowledgment: The authors thank the Central Service Laboratory at College of Education for Pure Science (Ibn Al- Haitham), University of Baghdad for providing the facilities to carry out this work. The authors would also like to thank Department of Chemistry for providing consultancies.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Khan N, Choi JY, Nho EY, Jamila N, Habte G, Hong JH, et al. Determination of minor and trace elements in aromatic spices by micro-wave assisted digestion and inductively coupled plasma-mass spectrometry. Food Chemistry. 2014;158:200–206.
2. Fraga CG. Relevance, essentiality and toxicity of trace elements in human health. Molecular Aspects of Medicine. 2005;26(4–5 SPEC. ISS.):235–244.
3. Smichowski P, Londonio A. The role of analytical techniques in the determination of metals and metalloids in dietary supplements: A review. Microchemical Journal. 2018;136:113–120.
4. Haque N, Islam MMT, Hassan T. Determination of Heavy Metal Contents in Frequently Consumed Fast Foods of Bangladesh. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences. 2019; 89(2):543–549.
5. Uddin J, Abdul A. Heavy metal contamination in water , soil , and vegetables of the industrial areas in Dhaka , Bangladesh. 2010;166(1–4):347–357.
6. Shim J, Cho T, Leem D, Cho Y, Lee C. Heavy metals in spices commonly consumed in Republic of Korea. Food Additives & Contaminants: Part B. 2019;12(1):52–58.
7. Olusakin O P, Olaoluwa J D. Evaluation of Effects of Heavy Metal Contents of Some Common Spices Available in Odo-Ori Market, Iwo, Nigeria. Journal of Environmental Analytical Chemistry. 2016;03(01):3–6.
8. Baig JA. Evaluation of Arsenic , Cadmium , Nickel and Lead in Common Spices in Pakistan. 2019;187(2):586–595.
9. Nkansah M, Amoako C. Heavy metal content of some common spices available in markets in the Kumasi metropolis of Ghana. American Journal of Scientific and Industrial Research. 2010;1(2):158–163.
10. Divrikli U, Horzum N, Soylak M, Elci L. Trace heavy metal contents of some spices and herbal plants from western Anatolia, Turkey. International Journal of Food Science and Technology. 2006;41(6):712–716.
11. Krejpcio Z, Król E, Sionkowski S. Evaluation of heavy metals contents in spices and herbs available on the Polish market. Polish Journal of Environmental Studies. 2007;16(1):97–100.
12. Inam F, Deo S, Narkhede N. Analysis of minerals and heavy metals in some spices collected from local market. IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS). 2013;8(2):40–43.
13. Ibrahim GI, Hassan LM, Baban SO. Effect of Heavy Metal Content of some Common Spices Available in Local Markets in Erbil City on Human Consumption. Rafidain Journal of Science. 2012; 23(3): 106–114.
14. Karadaş C, Kara D. Chemometric approach to evaluate trace metal concentrations in some spices and herbs. Food Chemistry. 2012;130(1):196–202.

Detection Malta Fever by Interferon-gamma and Steroid Hormone S Level

Ali Anok njum[1], Layla Saleh Abdul-Hassan[2] Ihasan A. Hashem[3]

[1]Assist Prof., [2]Lecturer, [3]Assistant lecturer/Al-Furat Al-Awsat Technical University/College of Health and Medical Techniques/kufa / Iraq

Abstract

Malta fever is one of the most common bacterial zoonosis, its causes abortion of pregnant women. Abortion is the chief obvious manifestation of *Brucella* infection. *Brucella* like better cattle placenta as a result of great concentration of erythritol sugar, whereas human placenta there is no erythritol sugar only steroid hormone, for this reason designed the our project . In this study, 100 aborted women were included , where referred to maternity and children hospital of Babylon province \ Iraq. Diagnosis of Brucella infection in these abortions was concentrated on serological and bacteriological technique. Serological studies included the use of RB and ELISA tests. Aggressive differences between RB and ELISA results have been shown. Brucella isolated and identified from aborted placenta and blood samples were 7 (7%) isolates from aborted women.

Hormonal assessment by Immunohistochemical technique in *Brucella* infected women, showed significant decrease in progesterone expression in comparison with that aborted due to other causes, in other hand *Brucella* infected women showed high expression in estrogen hormones . ELISA technique was the valuable serological test to confirm the diagnosis of brucellosis as compared with RB test.

Keyword: *Malta fever , Brucella , Interferon-gamma , Steroid hormone*

Introduction

Malta fever also called Brucellosis, is a zoonotic disease effecting humans and animals in many countries e.g, Mediterranean area, Iraq, middle east, India, and America(1) . Reports from the regions where *Brucella melitensis* infection is endemic, propose that there is an increased rate of abortion in asymptomatic pregnant women. The diagnostic method acknowledged to produce the best results in terms of specificity is the isolation of *Brucella* organisms from the suspected human , Different *Brucella* species are recognized as causative agents of brucellosis and some of them are acknowledged to be pathogenic to humans (2-8) . However, Because of the variety of the disease and its non-specific clinical manifestation, the clinical diagnosis of Malta fever remains a challenge. Malta fever (Brucellosis) mimics other infectious and non-infectious diseases, resulting in a delay in diagnosis or misidentification of the disease(9). The diagnosis is importantly dependent on a epidemiological history , clinical signs , patient's medical, biochemical testing , hematological, radiological examination and, necessarily, on *Brucella*-specific laboratory tests. Very important sides for cporrect and fast diagnosis are the disease-specific laboratory tests and knowledge of their weaknesses, proper analysis and correct assessment of their results (10). Rose Bengal (RB) is the main serological test used to identify antibodies against brucellosis. Because of the difficulties in the method and low sensitivity of the isolation methods, laboratory diagnosis relies largely on serological tests. The major antigens of *Brucella* being used in serological testing are the internal-cytosolic proteins and lipopolysaccharide (smooth-S LPS) (11). *Brucella* Lipopolysaccharide is a strong immunogen but its epitopes are the chief etiology of cross-reactions with other Gram-negative bacteria , a condition that creates evaluation more difficult(12). Cytokines have an important role in the pathogenesis of Malta fever and the Th1/Th2 balance may include in the resistance or susceptibility to the disease (13). As the pathogen is intracellular, Th1 and its linked cytokines

are responsible for control of the infection. Experimental studies showed that IFN-γ is essential for exclusion of *Brucella* and for host survival in case of virulent *Brucella* challenge [14].The aims of the present work were:

1- tropism of *Brucella* to uterus of women that it not has erythritol sugar

2- Evaluate steroid hormones level in placenta and blood is specific method to detection of Malta fever .

3- Evaluate interferon-gamma level in blood.

Materials and Methods:

Human brucellosis kit was provided by Elabscience /China. Gram's stain solution. Rose Bengal antigen was provided by Omega company, UK. antibrucella abortus , antibrucella melitensis and Monospeciic antiserum were supplied by Difco, USA.

Methods

Samples collection.

1-Clinical signs of aborted women were recorded by physician to show pregnancy period and sings of abortion.

2-Blood samples: Hundred blood samples from women were obtained. serum samples have been obtained for serological and immunological assessments using RB and ELISA tests.

3-Aborted Placentas:

Hundred placenta samples from aborted women have been obtained, at maternity and children hospital of Babylon province, placenta samples were cultured directly on *Brucella* selective medium and blood agar.

4-Immunohistochemistery for placenta specimens was performed as described by (Dako, UK), The expression of "estrogen and progesterone hormones were measured as the same scoring system" used by Mao *et al*., (15). The positivity of cells for expression of hormones were seen as brown staining. It was graded as four grad of the cells staining positive for hormones.

Score	0	1+	2+	3+	4+
Positive Cells	<10%	10-25%	25-50%	50-75%	>75%

5- IFN-gamma ELISA performed as described by (RayBiotech. Inc).

Statistical Analysis:

The results are expressed at percentage by Chi-square test, (SPSS) for Windows program was used to compare between the frequencies. Student t test was used to compare between means of groups. The significance was accepted as P value < 0.05 .

Results

Clinical signs in women included fever and bleeding, most women infected with *Brucella* were aborted at first stage of pregnancy .

Serological tests:

ELISA , RB test revealed that RB results were positive in 27 cases (27%). ELISA results were positive in 14 cases(14%) . according to Pearson Chi-Square test, the difference in RB and ELISA positive cases was significant (P<0.05) show in Table (1) .

Table(1) showed the diagnostic test of *Brucella* infected women

Blood samples	RB	%	Culture(+)	%	ELISA	%
100	27	27	7	7	14	14

Bacterial isolation & identification:

Out of the 100 aborted women, 7 (7%) were positive for culture, from 14 patient blood samples that positive for positive ELISA, 7 isolates were positive by culture, after 2-4 days the *Brucella* culture recognized on the basis of colonial morphology (translucent , round with pearly appearance).

Isolates from blood and placenta samples were Gram-negative, coccobacilli, arranged singly in short chain or small groups stained with modified ziehl-

neelsen stain , Biochemical test of *Brucella* show in table (2)

Table (2): Biochemical test of *Brucella*

	Test	**Results**
1	Haemolysis	-
2	macconkey agar	-
3	indol	-
4	MR-VP	-
5	gelatinase	-
6	Citrate utilization	-
7	urease	+
8	nitrate reduction	+
9	catalese	+

Steroid hormones assessment by immunohistochemistry assay:

Result of imunnohistochemical assay showed positive staining for progesterone hormone in placenta of aborted woman non infected with *Brucella* , compared with that of positive for *Brucella* infection which exposed low intensity for IHC staining of progesterone, the score differences were also seen in(table 3).

Table (3) : Existence of progesterone molecule in placenta of aborted women .(IHC assay)

Score	**Positive for Brucella infection**		**Negative for Brucella Infection**	
	NO	**%**	**NO**	**%**
1	3	42.58	0	0
2				
3	4	57.14	1	14.28
Tatal of negative score	*100%		14.28%	
4	0	0	1	14.28
5	0	0	5	71.42
Tatal of positive score	0%		85.71%	

*Significant ($p \leq 0.05$)

According to study the estrogen particles . there is obvious rise for estrogen stain for placenta tissue through *Brucella* infection . as determined by staining of biopsies , the immune staining of estrogen were positive at high level in 85.71% (6 out of 7) in *Brucella* infected patients, with highly statistical association ($p \leq 0.05$) between the infected & non infected groups (table 4).

Table (4) : Amount of estrogen molecules in placenta of aborted women (IHC assay)

Score	Negative for Brucella infection		Positive for Brucella Infection	
	NO	%	NO	%
1	0	0	5	71.42
2	2	28.57	1	14.28
3	5	71.42	1	14.28
Score ;1< 25% ; 2(25-74)% ; (75-100)%				

Interferon-gamma assessment by ELISA technique

Patients with Malta fever had significantly (P<0.05) higher serum levels of IFN-γ (175.078 ± 69.821pg/ml) compared to control group (39.358 ±29.847 pg/ml) show in (Figure 1).

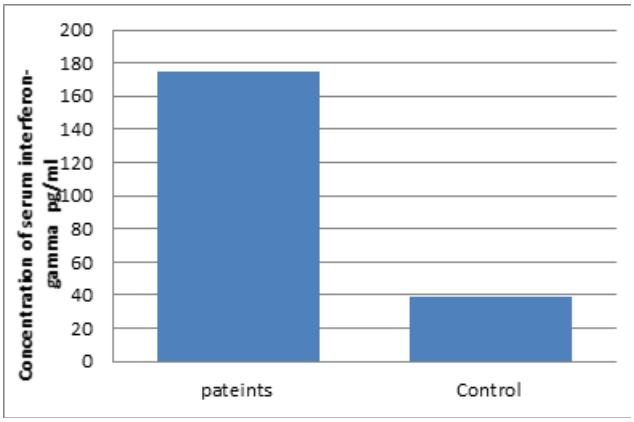


Figure 1 : Serum concentration of IFN-γ in patients and control

Discussion

RB gave positive result in aborted women but were negative for ELISA and bacterial isolation infection, because the RB test is rapid and screening test and may be give cross-reaction with other Gram negative bacteria example : *E.coli* O:157, *Yersinia enterocolitica* O:9, *Vibrio cholerae* O:1, *Salmonella spp., Francisella tularensis and Pseudomonas maltophilia,* and give false positive for RB [16].

Antibodies to *Brucella* appear in the serum within (1-2) weeks of infection. The initial response is the appearance of IgM isotype (which can be easily detected by RB) followed by a switch to IgG, after a while titers of both Immunoglobulins classes increase distinct most of the usual serological tests, ELISA is effective in distinguishing all immunoglobuline (antibodies) classes and sub-classes essential in diagnosis and appears to be the most sensitive serological test increase in IgG but not IgM. IgA titres roughly paralleled IgG titers[17]. ELISA using S-LPS Ag can be used to measure the development of immunoglobulin isotypes following infection and after treatment[18]. Steroid hormones concentration in aborted women positive for brucellosis were showed decrease in progesterone levels and increase in other hormones due to *Brucella* infection, aborted women negative for brucellosis notice significant increase in hormone , this certified that other cause of abortion may be not effect on progesterone synthesis.

Estimation of hormones are more effective method to conformation of *Brucella* infection, while other serological methods may be causes cross-reaction and false positive results.

There are two HSD3B1 proteins, labeled type I and type2 that are expressed by different genes and function in different regions of the body[19]. HSD3B1has too been shown to be there a highly specific and sensitive trophoblast-associated marker, also showed that expression of 3β-HSD in trophoblast more than 50% considered as positive cells for 3βHSD [20]. To form steroid hormones, the subsequent processing of pregnenolone requires enzymes related to smooth endoplasmic reticulum, such as 17 α-hydroxylase (P450c17α) and 3β-hydroxysteroid dehydrogenase/ isomerase (3βHSD) pregnenolone is converted to progesterone by the enzyme 3β-HSD, which in turn, is converted to androstenedione by the enzyme 17α-hydroxylase/C17, 20 – lyase (P450c17α) [21].

In this study, have high serum levels among patients with Malta fever compared with control. These results are in agreement with earlier studies [22] who reported height this cytokine in Malta fever patients.

Most studies specified that CD4+ T lymphocytes are the main producer of IFN-γ although other subsets such as "CD8+ Tlymphocytes, γδ T lymphocytes and NK cells also participate in the production of this cytokine [23]. As there are relatively high serum levels of IFN-γ, it indicates an enlarged number of CD4+ which, in turn, indicates a chronic infection" [24].

Conclusion

. In conclusion, placental immunohistochemical and assessments for steroid hormones (progesterone) and interferon-gamma have an efficient diagnostic values which can be included for confirmation of brucellosis .

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1- Ntirandekura, J. ; Ngowi, H. A.; Matemba, L. E. ; Kimera, S. I. and Karimuribo, E. D.: Knowledge, perceptions and practices regarding brucellosis in pastoral communities of Kagera Region in Tanzania, Journal of Advanced Veterinary and Animal Research, (2018) 5(3), 243-253 .

2- Aguero, F., Land, M.L., Ugalde, R.A., Garcia, E., Whole-genome analyses of speciation events in pathogenic Brucella. Infection and Immunity (2005). 73, 8353 – 8361

3- Whatmore AM, Davison N, Cloeckaert A, Al Dahouk S, Zygmunt MS, Brew SD, Schlabritz-Loutsevitch NE. *Brucella papionis* sp. nov., isolated from baboons (*Papio* spp.). International Journal of Systematic and Evolutionary Microbiology. 2014; 64:4120–4128

4- Musallam II, Abo-Shehada MN, Hegazy YM, Holt HR, Guitian FJ. Systematic review of brucellosis in the Middle East: disease frequency in ruminants and humans and risk factors for human infection. Epidemiology and Infection. 2016; 144(4):671-685.

5- Obonyo M, Gufu W. Knowledge, Attitude and practices towards brucellosis among pastoral community in Kenya, 2013. International Journal of Innovative Research and Development. 2015; 4(10):375–384.

6- Hosein, H.I., Rouby, S., Menshawy, A. and Ghazy, N. Seroprevalence of camel brucellosis and molecular characterization of *Brucella melitensis* recovered from dromedary camels in Egypt. *Res. J. Vet. Pract.,* (2016) 4(1): 17-24.

7- Hosein, H.I., Rouby, S.R., Menshawy, A. and AbdAl- Ghany, A.E. Sensitivity and specificity of the commonly used diagnostic procedures of bovine brucellosis. *Vet. Sci. Res. Rev.*, (2017) 3(3): 45-52.

8- Affi, M.M., Abdul-Raouf, U.M., El-Bayoumy, E.M., Montasser, A.M. and Mohamad, H.A. Isolation and biotyping of *Brucella melitensis* from Upper Egypt. *Report Opin*., (2015) 7: 79-85.

9- Acha, P. N. and Szyfer, B. Brucellosis in: zoonoses and communicable disease common to man & animals. 2nd edition P. 24-45. Pan American Health Organization (1987)..

10- Alton, G.G. *Brucella melitensis*. In: Nielsen, K.H.; Duncan, J.R. Animal brucellosis. Boca Raton: CRC. (1990). p.383-422.

11- Alton, G.G.; Jones, L.M.; Angus, R.D. and Verger, J.M. Techniques for the brucellosis laboratory. 1st ed. INRA. Paris. Ch.2 p. 114. (1988).

12- AL-Byatti, L.H. Bacteriological and Serological study on important causes of abortion in women with concerning on brucellosis. MSc. Thesis. Vet. Med coll. Baghdad. University/(2004).

13- Hamza, T.; Barnett, J. B. abd Li, B. Interleukin 12 a key immunoregulatory cytokine in infection applications. *Int. J. Mol. Sci.,* (2010). *11*: 789-806.

14- Ahmed, K.; Al-Matrouk, K. A.; Martinez, G.; Oishi, K.; Rotimi, V. O. and Nagatake, T. Increased serum levels of interferon-γ and interleukin-12 during human brucellosis. *Am. J. Trop. Med. Hyg.,* (1999). *6*: 425-427.

15- Mao, M.D., Robert ,J., Kurman, M.D. and IE-Ming ,S. HSD3B1 as a novel trophoblast-associated marker that assists in the differential diagnosis of trophoblastic tumors and tumorlike lesions. In The American journal of surgical pathology, (2008). 32, (2): 236-242

16- Araj, G. Human Brucellosis revisted., B. M. J. middle east Atlas, R. M. (1995). Principles of microbiology 1st ed. Unuversity of Lonisville, Kentuck, Mosby Year book inc: (2000). 648.

17. Buzgan,T., Karahocagil, M.K.,Irmak,H., Baran,A.I.,Karsen,H.,Evirgen,O.and Akdeniz,H. Clinical manifestations and complications in 1028 cases of brucellosis : a retrospective evaluation and review of the literature . International Journal of Infectious Disease , (2010).14:469

18- Cetin, O. ; Kurdoglu, Z. and Akdeniz, H. Effect of Brucellosis on Women's Health and Reproduction. International Journal of Women's Health and Reproduction Sciences , (2015): 3 (4) : 176–183.

19- Cross, J.C. (2002). Genetic insights into trophoblast differentiation and placental morphgenisis. Semin cell Dev. Biol. 11:105-113.

20- Halling, S.M., Peterson-Burch, B.D., Bricker, B.J., Zuerner, R.L., Qing, Z., Li, L.L., Kapur, V., Alt, D.P., Olsen, S.C., Completions of the genome sequence of *Brucella* abortus and comparison to the highly similar genomes of *Brucella* melitensis and *Brucella* suis. Journal of Bacteriology (2005). 187, 2715–2726.

21- Tuckey, R.C .(Progesterone synthesis by the human placenta. Placenta . (2005). 26: 273–281.

22- Rafiei, A.; Ardestani, S. K.; Keyhani, A.; Mohraz, M. and Amirkhani, A. Dominant Th1 cytokine production in early onset of human brucellosis followed by switching towards Th2 along prolongation of disease. *J. Infect.,* (2006). *53*: 315-324.

23. Baldwin, C.L. and Goenka, R. Host immune responses to the intracellular bacterium Brucella: does the bacterium instruct the host to facilitate chronic infection? Crit Rev Immunol. 2006;26(5):407-42

24. Slavic, V.; Stankovic, A. and kamenov, B. The role of interleukin-8 and monocyte chemotactic protein-1 in rheumatoid arthritis. Med. Biol., (2005). 12: 407–442.

Hepatic Toxicity in Patients with Rheumatoid Arthritis and Psoriasis Taking Methotrexate Therapy

Haider Hamza Omran[1], Basim Mohammed Mothlum[1]

[1]Assist Prof./FICM. Internal medicine Merjan Teaching Hospital-Ministry of Health/Iraq

Abstract

Background: We had made a study to demonstrate the adverse hepatic effects of MTX in patients taking MTX for treatment of RA and psoriasis taking in consideration the following variables: BMI, gender, cumulative dose, age, weekly dose, duration of treatment, serum level of cholesterol and creatinine.

Patient and method: We had a prospective study of 85 patients with RA and 50 patients with psoriasis. All patients were analyzed by history, clinical examination and investigations in the form of liver enzymes, blood sugar, serum cholesterol, serum creatinine, HBS Ag and anti HCV antibody. Persistently elevated level of liver enzymes 2 to 3 times the upper limit of normal on two occasions 3 months apart indicate hepatic toxicity

Results: We found that 7 patients with psoriasis and 6 patients with RA have significant elevated liver enzymes which reflect MTX hepatotoxicity

Conclusion: Our study show that patients with psoriasis at significantly greater risk of elevated liver enzymes than patient with RA (14% and 7% respectively) were gender, BMI, cumulative dose, weekly dose and serum cholesterol level are risk factors for hepatic toxicity due to MTX therapy

Key words: *Hepatic Toxicity, MTX therapy, Arthritis*

Introduction

Methotrexate is a folic acid antagonist that inhibits dihydrofolate reductase. DNA synthesis is inhibited as the concentration of thymidine and purines falls after treatment with methotrexate.(1) The relevant targets of low dose methotrexate have not been defined with precision, but an attractive candidate is the enzyme 5- amino-imidazole -4- carboxamide ribonucleotide (AICAR) transformylase. Inhibition of AICAR transformlase leads to accumulation of AICAR, which in turn stimulate the extracellular release adenosine which has a number of anti-inflammatory and immunomodulatory effects that may contribute to the therapeutic effect of methotrexate.(2) Recently, in vitro studies showed that methotrexate was 10-100 times more effective at inhibiting the proliferation of lymphoid cell lines than cultured kerationocytes, suggesting that lymphoid cells may be a more important cellular target than epithelial cells in psoriasis and also inhibits polymorpho nuclear leukocyte chemotaxins.These actions may explain its clinical effect.(3)

Methotrexate is now the most widely used disease modifying antirheumatoid drug (DMARD) in the developed world. It was first used in the treatment of psoriasis and psoriatic arthritis in 1951, (4) and has been shown to be of clinical benefit in this condition. (5, 6)

Methotrexate has been used for the treatment of diseases characterized by inflammation or cellular proliferation. In 1985, the first randomized placebo-controlled trials were published that demonstrate the short term efficacy of low dose weekly methotrexate in Rheumatoid arthritis. (7-8)

Comparative studies of methotrexate with azathioprine (9), gold sodium thiomalate(10) and cicolosporine(11) in the treatment of Rheumatoid arthritis show that methotrexate is well tolerated, with retension rates between 93% and 95%.

Oral therapy is given once a week. Daily dose schedules are dangerous and have been abandoned. The oral triple-dose regimen is the most common method

used. A dose is taken at 12 hours intervals during a 36 hours period once each week. An initial test dose of 2.5 to 5mg is given and complete blood cell counts and liver function tests are obtained once week later. Maintenance doses should be achieved by gradual increases of 2.5-5mg per week up to 20mg/week [12].

The most common adverse effects are gastrointestinal, such as anorexia, nausea, vomiting, stomatitis and diarrhoea. Central nervous system toxicity including headache, dizziness, fatigue and mood disturbance may occur. Haematological toxicity is not common, and although all types of cytopenia has been documented. Pulmonary complications such as methotrexate pnemonitis may be linked to risk factors as increased age, diabetes mellitus and pre-existing pulmonary diseases [13]. It is now thought that the frequency of severe liver disease occurring in rheumatoid arthritis patients receiving long term methotrexate is not high; the incidence of mild fibrosis has been reported as being between 0% and 2% [14-15].

Base line monitoring include history, physical examination, complete blood count, platelet count, renal function test, liver chemistry including (AST, ALT, alkaline phosphate, albumin) and viral serology mainly for hepatitis B, C and HIV antibody and chest radiograpghy to exclude pulmonary fibrosis.Follow up monitoring include monthly complete blood count and differential, platelet count for the first and second dose then every two months, liver chemistries monthly or every two months, and renal function test including blood urea, serum creatinine at three to four months interval.

Persistently elevated liver enzymes more than two times would preclude further therapy. Although combined sensitivity of liver enzymes for detecting a significantly abnormal liver biopsy is 86%, whereas the predictive value of negative test result 93% [16], liver biopsy remain more reliable predicator of liver damage. Current study aimed to Assess the prevalence of liver enzyme abnormality in patients with RA and psoriasis taking MTX therapy and to identify the possible risk factors for MTX induce hepatotoxicity in these patients.

Patients and Method

Eighty five patients with rheumatoid arthritis diagnosed according to American college of rheumatology criteria [17] and 50 patients with psoriasis diagnosed by the presence of psoriasis with or without seronegative peripheral arthritis [18], underwent a prospective study

The study was performed in Marjan Teaching Hospital in Babylon for outpatient clinic of rheumatological and dermatological disease.

After taking the verbal consents of the patients, full history regarding age, gender, MTX dose per week, MTX duration, and cumulative dose defined as dose per week multiplied by duration of treatment, MTX adverse effects mainly the gastrointestinal problems (nausea, vomiting, abdominal pain and anorexia). Oral MTX therapy and folic acid supplement prescribed for all patients (5mg once daily). drug history concentrated on (NSAIDs, statins, cordaron, oral hypoglycemic drugs, psoraline+UVA treatment, gold, oral contraceptive pills, long term steroid and extreme obesity), any patient on these drugs for the last month were excluded, history of alcoholism so any patient drunk alcohol in the last 5 years were excluded from the study

History of other comorbid disease like congestive heart failure, chronic viral hepatitis, autoimmune hepatitis, Wilson's disease, chronic renal failure and diabetes mellitus were excluded from the study. Clinical examination include jaundice, ascites, organomegaly was performed, BMI represent the height and weight recorded as Wt. in Kg / Height in m^2 were 18 - 25 considered normal, 25 - 29.9 considered overweight, 30 – 39.9 considered obese and >40 considered extreme obesity[19] . Investigations in the form of liver enzymes (normal reference values for ALT, AST <20 U/100 ml, normal reference for ALP, 85 U/100 ml) done by colorimetric method, random blood sugar normal reference value <11.1 mmol/L by glucose oxidase method, cholesterol level normal reference value <5.2 mmol/L by cholesterol oxidase method, serum creatinine normal reference value <124µmol/L by alkaline picrate with Deprot method and viral serology for HBS Ag, anti HCV Ab. By bioelisa color method (direct immune enzymatic method).Persistently elevated level of liver enzymes 2 – 3 times the upper limit of normal on two occasions 3 months apart was indicate hepatic toxicity[20]

Statistical Analysis

Statistical significance of liver enzymes between patients with RA and psoriasis, and each risk factors were assessed by t-test and of the proportion, were p-value < 0.05 indicate Statistical significance the results were expressed as tables

Results

The average age of patients with RA was 48 years and for patients with psoriasis was 50 years, female outnumbered male in RA group were 56 to 29 and for psoriasis group, the female outnumbered male 23 to 27.body mass index in RA group was 24kg/m^2 and for psoriasis group was 27kg/m^2.The average duration of MTX therapy was 4 years in RA group and 3 years in psoriasis group. The average dose of methotrexate therapy in RA group was 10 mg/week and for psoriasis group was 15 mg/week [Table 1].

Regarding gastrointestinal symptom as nausea and vomiting were found in 20 patients (23.5%) with RA and in 10 patients (20%) with psoriasis [Table 2], statistically not significant.

Sustained rise in liver enzyme were seen in 6 patients (7%) in RA group while in 7 patients (14%) in psoriasis group [Table 3] which was significantly significant. The gender ,liver enzyme abnormalities were found in 2 males (33.3%) and in 4 females (66.6%) in RA group while 4 males (57.1%) and in 3 females (42.8%) in psoriasis group [Table 4],statistically not significant. The age, the average age in RA was 46 and 52 years in psoriasis group [Table 5], statistically not significant. BMI, in RA group was 23 kg/m^2 and in psoriasis group was 28 kg/m^2 [Table 6], statistically significant.

(Table 1) Demographics of study population

Variable	RA	Psoriasis
Total number of patients	85	50
Average age (year)	48(15-65) ± 12	50(18-65) ±13
Female	56(65%)	23(46%)
Male	29(35%)	27(44%)
Average of BMI Kg/m^2	24(17-30) ±2	27(20-35) ±4
Average dose of MTX Mg/week	10(5-10) ±3	15(10-20) ±4
Average duration of treatment (year)	4(1-6) ±2	3(1-5) ±2

(Table 2) Predictive value of gastrointestinal symptoms in RA and psoriasis

Variable	RA	Psoriasis	P-value
Total no. of patients	85	50	Not sign.
Nausea and vomiting	20(23.5%)	10(20%)	

(Table3) Predictive value of elevated liver enzymes in RA and psoriasis

Variable	R.A	Psoriasis	p-value
Total no. of patients	85	50	<0.05 (Sign.)
Elevated liver enzymes	6(7%)	7(14%)	

(Table 4) Predictive value of gender in RA and psoriasis

Variable	RA	Psoriasis	P-value
No. of patients with elevated liver enzymes	6	7	Not sign.
Female	4(66.6%)	3(42.8)	
Male	2(33.3)	4(57.1%)	

(Table5) Predictive value of age in RA and psoriasis

	Group	No. of patients with elevated liver enzymes	Average of age(year)	p- value
A G E	R.A	6	46(24-60)±13	Not sign.
	Psoriasis	7	52(35-65)±12	

(Table 6): Predictive value of BMI in RA and psoriasis

	Group	Number of patients with elevated liver enzymes	Average BMI	P-value
B M I Kg/m2	R.A	6	23(21-25)±2	<0.05 (Sign.)
	Psoriasis	7	28(21-38) ±3	

Discussion

The study showed that gastrointestinal problems like nausea, vomiting in RA group was 23.5% and in psoriasis group was 20% which was statistically not significant, in agree to study in 2006, where nausea and vomiting in psoriasis group (9.8) and in RA group was 13% which also statistically not significant[20]. A significant rise in liver enzymes were seen in 6 patients (7%) with RA group and in 7 patients (14%) with psoriasis group, our study agrees with study in 2006 showed that significant rise in liver enzymes in psoriasis group (14.5%) and 7.5% in RA group.[20] The study showed that the gender in RA group and psoriasis group not significantly correlated with hepatic toxicity in contrast to other study, one in 2006[20] other in 2004[34], both showed male in psoriasis group significantly more affected than male in RA group. The study showed no significant correlation of both the age, duration of MTX treatment with the level of liver enzymes abnormality in agree to a study in 2006[21] and other in 2004[22] The study showed that both BMI, cumulative dose, were significantly correlated with hepatic toxicity in psoriasis versus RA group in agree with study in 2004[23] The study showed a significant association between weekly dose of MTX with hepatic toxicity in psoriasis group versus RA group in contrast to study in 2004[22] The study showed a significant association between serum cholesterol level and hepatic toxicity in psoriasis group versus RA group in agree to study in 2006[24] but in contrast to previously reported studies [25--26] were total cholesterol level did not correlate with hepatic toxicity. This study showed no significant association between serum creatinine level and hepatic toxicity in psoriasis group versus RA group, a study in 2004[22] show that renal impairment, diabetes and obesity were significantly correlated with hepatic toxicity Current study show that liver enzymes (transaminases and ALP) both increased

in both groups (RA and psoriasis), although increase level of ALP more correlated with hepatotoxicity[27] .The study recommended encouragement of life style modification for patients who are overweight or obese with psychological support especially for patients with psoriasis,Viral serology for HBS Ag and HCV as baseline because MTX therapy in patient with positive serology may enhance viral replication and result in fulminant hepatitis and Baseline lipid profile as hyperlipidaemia is an independent risk factor for MTX hepatotoxicity

Conclusion

MTX related hepatic toxicity was more significant in patient with psoriasis than patient with RA. BMI, cumulative dose, weekly dose and serum cholesterol level are risk factor for hepatotoxicity in psoriasis group than in RA group.

Acknowledgement: The authors would like to thank the internal medicine department in Marjan Teaching Hospital in Babil/Iraq.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1- Thomas, P. Habif : Clinical Dermatology 4th ed. Dartmouth medical school, Han over, NH, USA, 2004, p. 229-233.

2- Danial , P. Stites : medical immunology 9th ed. Mc Graw- Hill companies, USA, 2001, chapter 53, P. 744-746.

3- C.E.M. Griffiths, R.D.R. and et al, Rook : Text book of Dermatology 8th ed. USA, 2006, psoriasis chapter 35. P. 37-38.

4- Gubner R, Augusts , Ginsberg v. Theraputic supprellion of tissues reactivity : effects of aminopetrin in R.A and psoriasis. Amj med 1951;211:276-82.

5- EspinzulR , Zakraoul , Carman G,etal. Psoriatic arthritis : clinical response and side effects to methotrexate therapy. J Rhenmatol 1992 ; 19 : 872-7.

6- Kersley GD. Amethopterine (methotrexate) in correctre tissue dican , psoriasis and poly arthritis. Ann Rheum Dis 1998 ; 27 : 64-6.

7- Weinblatt JE, coblynjs , Fox Dam etal. Efficacy of low dose MTX in Rhenmtoid arthritis . NEnglj med 1988;312:818-22

8- Williams HJ, wilkens Rf, Samuelson Jr Co, etal. Comparsion of low dose oral pulse methotrexate and placbo in the treatment of rhenmatiod arthisite : acontrolled clinical trial. Arthristis rheum 1985;28:721-30.

9- Jenrissen mec, Boerbooms AmT, van de putte LBA,etal. MTX versus azathioprim in the treatment of R.A: a forty-eight week randomized donzle blind trial Arthritis Rheum 1991 ; 34 : 961-72.

10- Raur, Herbon G, Karger T ,etal. A double blind randomized parallel trial of intramnsculer mathoterxate and gold sodium thiomalate in early erosive rhenmatois arthritis. J Rhenmatol 1991 ; 18 : 382-33.

11- Cohens, Rutsteinj , Luggen M,etal. Comparison of safety and efficancy of cycloporin A and methotrexate in refractory rehnmatiod arthritis : arandomized , multicentered placebo- controlled trial (abstract). Arthritis Rheum 1993 ; 36 : 556.

12- Roenigk HH, Auerbach R, maibach HI et al. Methotrexate in psoriasis : revised guidelines. Jam Acad Dermatol 1988 ; 19 : 145-6.

13- Alcron Gs. Kermer jm, macaluson ,etal. Methotrexate lung toxicity in R.A: a multicenter case control study (abstract). Arthritis Rheum 1995 ; 38 : S 205.

14- Phillips CA, cera pj , mangan TF ,etal. Clinical liver disease in patients with R.A taking MTX, J Rheumatol 1992 ; 19 : 229-33.

15- Erikson AR, Reddy V, Vogelgesang SA, etal. usefulness of American college of Rheumatology recommendations for liver biopsy in MTX – treated R.A patients . Arthritis Rhenm 1995 ; 38 (8) : 1115-9.

16- O' connor GT, olmstead EM and et al . Detection of hepatoxcity associated with MTX therapy for psoriasis. Arch Dermatology 1989, 125: 1209-17.

17- Arnett Fc, Edworthy Jm, Bloch DA etal. The American Rheumatism Association 1987 revised criteria for the classification of rhumtoid arthritis. Arthritis rheum 1988 mar ; 31 (3) : 315-24.

18- Michet Jm . psorinatic arthritis. In : Kelley WN , Hawis ED Jr, Ruddys , etal, editors . text book of rheumtology. Philadelphia (PA) : WB sanm ders , 1993 : 974-84.

19- P. HANLON, M. Byers et al ; Environmental and nutritional factors in disease : Davidson's principle and practice of medicine. Churchill living stone, London 20th ed. 2006, part 1, chapter 5, p. 113.

20- Lindsey Tilliny ; sne Towosend ; Joel DAVID. FROM CLINICAL Drug investigation. Methotrexate and hepatoloxicity in Rheumatiod arthritis and psorintic arthritis, 2006; http: view article/ 528/88.

21- Vera M.R. heyclendael , MD, ph and etal. Analysis of risk factors in psoriatic patients with methotrexate-induced increases in transaminase levels, Vol 145 u, oct 2004

22- Lindsay K, Gough A, etal. The incidence of methotrexate induced hepatoxicity in psoriatic arthritis. 34th Annal European society for Dermatology logical Research (ESDR) meeting, 9-11 september 2004, Vienna, Austria.

23- Aithal GP, Hangk B, et al. Monitoring methotrexate-induced hepatic fibrosis in patient with psoriasis. Aliment pharmacol ther. 2006 feb 15 ; 19 (4) : 391-9.

24- Mallbris L, Granath F, Hamsten A, stablem. Psoriasis associated with lipid abnormality aton set of skin disease. Jonrnal of the American Academy of dermatology. 2006 ; 54(4) : 614-621.

25- Mallbris L, Akreo, Granath F, et al. Increased risk for cardiovascular mortality in psoriasis in patients but not out patients. Eur J Epidemiol. 2004 ; 19 : 225-230.

26- Cimsit G, orem A, deger o ,etal. The varintion of serum lipoprotein (a) level with disease activity in psoriasis. Br J dermatol. 1988; 138 : 917-919.

27- M.G.C. Dahi , M.M.Gregorg , and p.j. schemer liver damage due to methotrexate in patients with psoriasis. Journal list. Br med J. v.l (5750); mar 20, 1971.

Scolicidal Activity of Zirconium Oxide (ZrO_2) nanoparticles Against Protoscolices of Hydatid Cysts

Amenah Abdul-Jabbar Ibrahim

University of Baghdad, College of Education for Pure Science/Ibn Al-Haitham, Department of Biology /Iraq

Abstract

Hydatidosis is a sickness that affects human and farm animals. This disease is deemed as a public health problem in different regions of the world until nowadays. Surgical overlaps is the best way to treat the disease, while the risk of surgery lies in the possibility of cyst rupture and leakage of protoscolices and the recurrence of infection again, this prompted researchers to use scolicidal agents before surgery such as ethanol, plant extracts, to reduce parasite spread and recurrence of infection, recently researchers have been using nanoparticles as a scolicidal agent, like gold nanoparticles, silver nanoparticles, selenium nanoparticles, and others. This research aims to evaluate the fatal effect of zirconium oxide (ZrO_2) nanoparticles to protoscolices of hydatid cysts. The Protoscolices were collected from sheep livers infected with hydatid cyst disease. The protoscolices were treated with different concentrations (250, 500, 1000, 2000, and 4000 µg/ml) of ZrO2 NPs. The viability of protoscolices was determined by using an eosin staining method after 15, 30, and 60 min. The results showed that the concentrations of 1000, 2000, and 4000 µg/ml were significantly effective in the killing of protoscolices after 60 min., where the fatality rate of protoscolices was 49.6%, 52.7%, and 53.1% respectively when compared with the control group 38.5% ($p<0.05$).

Key words: *ZrO_2, Zirconium oxide nanoparticles, protoscolices, Scolicidal, hydatidosis.*

Introduction

Echinococcosis is a zoonosis, including two types of hosts, definitive (carnivores) and intermediate (wide range of mammalian species). The parasites of genus *Echinococcus* are small cestodes (1-6 mm) [(1)]. The metacestodes of *E. granulosus* are cysts of different sizes filled with clear liquid and are called hydatids. The first discovery of hydatid disease dates back to the time of Hippocrates [(2)]. Cystic echinococcosis (CE) is a growing health problem in various parts of the world, including the Middle East [(3)]. The liver is the first organ in which the parasite settles and develops into the larval stage (hydatid cyst) [(4)]. The germinal inner layer of hydatid cyst represents the origin of protoscolices, which are the infective form to the definitive host, and the source of secondary infection when naturally or experimentally released within mammalian tissues or peritoneal cavity [(5, 6)]. Surgery and/or chemotherapy are a common treatment for CE, chemotherapeutic of this disease has been sophisticated in several animal model studies, and both albendazole and mebendazole are considered to have identical efficiency [(7)]. Surgery is used in special cases depending on the characteristics of cysts, such as large cysts that contain multiple daughter cysts, single superficial cysts at risk of rupture, and cysts interlaced with biliary tract. PAIR (Percutaneous, Aspiration, Injection and Re-aspiration) is an alternative way to surgery, with minimum risks [(8)]. Efforts were dedicated to finding out new protoscolicidal materials from plant sources, those efforts concentrated on some plant extracts that showed high effectiveness against CE [(7)]. Due to the unavailability of effective treatment for CE, there is an urgent need to find this treatment, so that the nanotechnology-based materials may be useful in the cure of diseases [(9)]. Nanotechnology is a technique that is concerned with the development and use of chemicals, devices, and systems of unfamiliar characteristics because of their small size (1-100 nm), in addition to their unique physicochemical properties which had great importance in many fields of research, including science and medicine [(10)].

ZrO2 NPs have been studied extensively because of their unique mechanical, thermal, optical, and electrical properties [(11)]. They have broad applications because

of remarkable biocompatibility, high strength, and low cost [12]. ZrO2 NPs are also able to possess noticeable antimicrobial properties [13].

Materials and Method

1- Collection of protoscolices.

Samples of hydatid cysts were collected from livers of naturally infected sheep in a slaughterhouse in Baghdad/Iraq. The hydatid fluid was withdrawn from the cysts by using a 20 ml syringe and transferred into flasks. The fluid was left for enough time to enable all protoscolices to precipitate. The germinal layers of the cysts were also collected and placed in a petri dish and washed very well to assemble all protoscolices. The protoscolices were suspended with normal saline. The viability of protoscolices calculated according to the method of of Smyth & Barrett [14].

2- Preparation of nanoparticles suspension.

Zirconium nanoparticles (29.8 nm) were purchased. Jeng & Swanson [15] method was adopted to prepare nanoparticles suspension with modulation, where 0.4g of nanoparticles were weighed and suspended in 100 ml of distilled water and sonicated for 20 minutes before use. Five concentrations of the ZrO_2 NPs (250, 500, 1000, 2000, 4000) µg/ml were prepared according to the method of Napooni et al. [16] with some modifications.

3- Solicidal activity of ZrO_2 nanoparticles.

The efficiency of five concentrations (250, 500, 1000, 2000, 4000) µg/ml of ZrO2 NPs were tested against protoscolices of hydatid cysts at different exposure times (15, 30, 60) min. 0.5 ml of protoscolices suspension (1000 ps) were mixed with 0.5 ml of each concentration of nanoparticles, then incubated at 37°C for 15, 30 and 60 min. Then the viability of Protoscolices calculated after exposure to each concentration/time.

4- Statistical analysis

The data were analyzed by using statistical software package IBM SPSS (version 25). The differences between experimental groups and control groups estimated by using ANOVA and T-tests at a significance level of 0.05.

Results

Fatality rate (%) of protoscolices after exposure to ZrO2 NPs at various concentrations following various exposure times as shown in Figure 1.

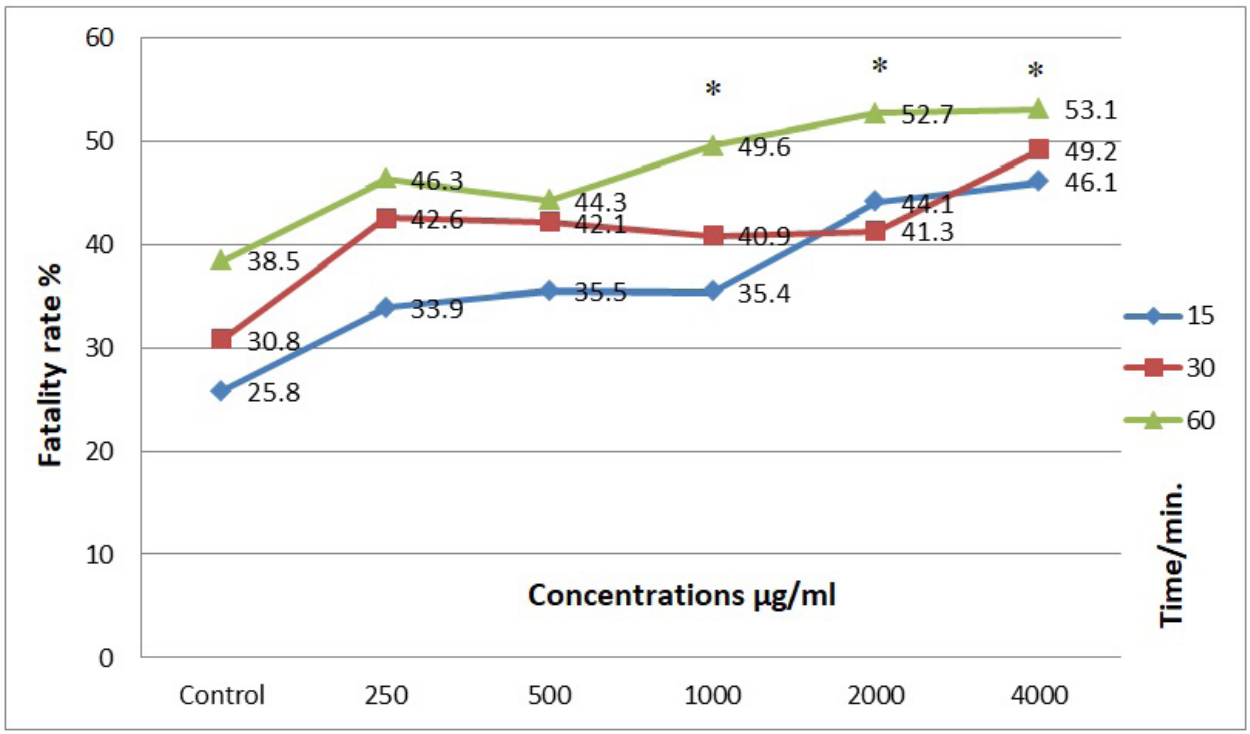


Figure 1: Fatality rate (%) of protoscolices after exposure to ZrO2 NPs at various concentrations following various exposure times.

* The difference between the experimental group and control group is statistical significance ($p<0.05$)

Discussion

The results of this study showed that the highest fatality rate of protoscolices was 49.6%, 52.7%, and 53.1% when treated with ZrO2 NPs suspension at concentrations 1000, 2000, 4000 µg/ml, respectively, after 60 min., and the difference between these groups and control group (38.5%) was statistically significant ($p<0.05$). Because of the importance of nanoparticles in medicine and science, the researchers studied its effects against some harmful organisms, especially bacteria and parasites, like the studies of Yalcinkaya et al. [17] and Kumaresan et al. [18], which proved the great effect of ZrO2 against *E. coli*, *Bacillus subtilis*, *Salmonella typhi*. Rahini et al. [19] studied the effect of green synthesis silver nanoparticles against protoscolices, and they found that the highest mortality rate was 90% (0.15 mg/ml, 120 min.). While the highest mortality rate was achieved when using the green synthesis gold nanoparticles (94%, 0.3 mg/ml, 120 min.) [20]. Naponi et al. [16] have also used gold nanoparticles at the same concentration for the current study and after 5, 10, 20, 30, and 60 min., they proved that all concentrations had a potential effect against protoscolices, while the highest mortality rate reached 76% (4000 µg/ml/ 60 min.).

Conclusion

It was concluded from this study that the ZrO2 was effective in the killing of the protoscolices of hydatid cysts, and the highest mortality rate reached 53%. It can be used other concentrations to elevate the efficiency of ZrO2 or combine these nanoparticles with plant extracts or drugs. It is suggested to study the effect of ZrO2 against hydatid disease in vivo, to ensure its safety and possible use as a treatment against this disease in the future.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1- Ciobanca, P.T. & Junie, M.L. Serological diagnosis and its applicability to the prophylaxis and therapy of hydatid cyst in human patients. Sci. Parasitol., (2011). 12(1): 39-46.

2- Rollinson, D. & Stothard, J.R. Historical aspects of Echinococcosis. In: Eckert, J. & Thompson, R.C.A. (Eds.). Advances in parasitology *Echinococcus* and Echinococcosis, Part A. Elsevier Academic Press, UK. (2017). 1-64pp.

3- Galeh, T.M.; Spotin, A.; Mahami-Oskouei, M.; Carmena, D.; Rahimi, M.T.; Barac, A.; Ghoyounchi, R.; Berahmat, R. & Ahmadpour, E. The seroprevalence rate and population genetic structure of human cystic echinococcosis in the Middle East: A systematic review and meta-analysis. Int. J. Surg., (2018). 51: 39-48.

4- Malik, A.A. & Ul Bari, S. Human abdominal hydatidosis., Springer, Singapore, (2019). 103pp.

5- Galindo, M.; Gonzalez, M.J. & Galanti, N. *Echinococcus granulosus* protoscolex formation in natural infections. Biol. Res., (2002). 35(3-4):365-371.

6- Dı´az1, A.; Casaravilla, C.; Irigoı´n, F.; Lin, G.; Previato, J.O. & Ferreira, F. Understanding the laminated layer of larval Echinococcus I: structure. Trends Parasitol., (2011). 27(5): 204-213.

7- Shnawa, B.H. Advances in the use of nanoparticles as anti-cystic echinococcosis agents: a review article. J. Pharm. Res. Int., (2018). (24(1): 1-14.

8- Tersigni, C.; Venturini, E.; Montagnani, C.; Bianchi, L. Chiappini, E. De Martino, M. & Galli, L. Should pediatricians be aware of cystic echinococcosis? a literature review. J. Pediatr. Gatr. Nutr., (2019). 68: 161-168.

9- Rai, M.; Ingle, A.P.; Paralikar, P.; Gupta, I; Medici, S. & Santos, C.A. Recent advances in the use of silver nanoparticles as antimalarial agents. Int. J. Pharm., (2017). 526(1-2):254-270.

10- Packer, L. & Cadenas, E. Biomedical Application of Nanoparticles. In: Santamaria, A.B. (Ed.). Nanoparticle world history and introduction to their diversity in medicine. CRC Press Taylor & Francis Group. Boca Raton London NewYork, (2018). 328pp.

11- Ashkarran, A.A.; Aghigh, S.M.; Afshar, S.A.A.; Kavianipour, M. & Ghoranneviss, M. Synthesis

and characterization of ZrO2 nanoparticles by an arc discharge method in water. Synth. React. Inorg. M-Org. Nano-M. Chem. (2011). 41: 425-428.

12- Fathima, J.B.; Pugazhendhi, A. & Venis, R. Synthesis and characterization of ZrO2 nanoparticles-antimicrobial activity and their prospective role in dental care. Microb. Pathog., (2017). 110: 245-251.

13- Gowri, S.; Gandhi, R.R. & Sundrarajan, M. Structural, Optical, Antibacterial and Antifungal Properties of Zirconia Nanoparticles by Biobased Protocol. J. Materials Sci. Tech., (2014). 30(8): 782-790.

14- Smyth, J.D. & Barrett, N.J. Procedures for testing the viability of human hydatid cysts following surgical removal, especially after chemotherapy. Trans. R. Soc. Trop. Med. Hyg., (1980). 74(5): 649-652.

15- Jeng, H.A. & Swanson, J. Toxicity of metal oxide nanoparticles in mammalian cells. J. Environ. Sci. Health Part A, (2006). 41: 2699-2711.

16- Napooni, S.; Arbabi, M.; Delavari, M.; Hooshyar, H. & Rasti, S. Lethal effects of gold nanoparticles on protoscolices of hydatid cyst: in vitro study. Compar. Clin. Pathol., (2018). 28(1): 143-150.

17- Yalcinkaya, F. & Lubasova, D. Quantitative evaluation of antibacterial activities of nanoparticles (ZnO, TiO2, ZnO/TiO2, SnO2, CuO, ZrO2, and AgNO3) incorporated into polyvinyl butyral nanofibers. Polym. Adv. Technol., (2017). 28: 137-140.

18- Kumaresan, M.; Vijai Anand K.; Govindaraju K.; Tamilselvan S. & Ganesh Kumae, V. Seaweed Sargassum wightii mediated preparation of zirconia (ZrO2) nanoparticles and their antibacterial activity against grampositive and gram negative bacteria. Microb. Pathog., (2018). 124:311-315.

19- Rahimi, M.T.; Ahmadpour. E.; Esboei, B.R.; Spotin, A.; Koshki, M.H.K.; Alizadeh. A.; Honary, S.; Barabadi, H. & Mohammadi, M.A. Scolicidal activity of biosynthesized silver nanoparticles against Echinococcus granulosus protoscolices. Int. J. Surg., (2015). 19: 128-133.

20- Barabadi, H.; Honary, S.; Mohammadi, M.A.; Ahmadpour, E.; Rahimi, M.T.; Alizadeh, A.; Naghibi, F. & Saravanan, M. Green chemical synthesis of gold nanoparticles by using Penicillium aculeatum and their scolicidal activity against hydatid cyst protoscolices of Echinococcus granulosus. Environ. Sci. Pollut. Res. Int., (2017). 24(6):5800-5810.

Molecular Study of *Fimh* Gene in *Klebisella Pneumonia*e Isolated From Urinary Catheter Patients

Israa Abdul Ameer Al-Kraety[1], Zahraa Hameed Oda Alquraishi[2], Aqeel A Alsadawi[3]

[1]Lecturer Department of Medical Laboratory Techniques, Faculty of Medical and Health Techniques, University of AlKafeel, Najaf, Iraq, [2.] Lecturer College of health & Medical tech., Al-Furat Al-Awsat Technical University, Kufa, Iraq, [3]Assistant lecturer Medical Laboratory Techniques Department, College of Medical Technology, The Islamic University, Najaf, Iraq

Abstract

This study aimed to isolate and diagnose *K. pneumoniae* from clinical specimens of urine from urinary catheterized patients and molecular Detection of *FimH* fimbrial adhesin in *Klebisella pneumonia* in Najaf governorate from October 2018 to March 2019, which includes 40 clinical specimens (urine). The diagnosis of *K. pneumoniae* isolates was based on culture and biochemical characteristics as an initial diagnosis. The final diagnosis by the Vitek-2 compact system is automated besides the use of PCR technique to detect on fimH fimbrial adhesion gene.

The biochemical results showed that 40/40 isolates gave positive result of *K. pneumoniae*.These results were confirmed by Vitek showed that 40/40 were positive for *K. pneumoniae* isolated and PCR technique by using *fimH* gene where 14/40 were positive for *K. pneumoniae* isolated from urine urinary catheterized patients.

The study, which was conducted in the diagnosis of bacteria, concluded that the technique of compact Vitek-2 automated. The ability of bacteria to stick to the formation of biofilm was investigated by phenotypic method.

***Keywords**: K. pneumonia, fimH gene, urinary catheterized.*

Introduction

Catheter-associated urinary tract infections (CAUTIs) are most frequent as a nosocomial infection with increased patient morbidity and health care costs. *Klebsiella pneumoniae* is a prominent opportunistic pathogen causing infection in 10% of the patients with urinary catheters. The catheter insertion provides site for bacteria attachment that is typical in Gram-negative enterobacteria [(1, 2)]. Furthermore, about 30% of *K. pneumoniae* isolates are resistant to broad-spectrum antibiotics with many virulence factors that have been identified . Fimbrial adhesins play an important role in the bacteria pathogenicity that facilitate adherence to specific tissue surfaces. Type 1 fimbriae, especially FimH subunit, found in many members of Enterobacteriaceae and play an important role in UTI [(3-5)]. Fimbriae are encoded by Fim gene cluster containing all fimbrial structure genes coding for repeating FimA subunits with an adhesin molecule (FimH) at the tip [(6)].

Material and Method

Patients and clinical specimens

A total of 40 urine samples were collected from urinary catheterized patients from different hospitals in Al- Najaf provenance from Oct. 2018 to March 2019.

Urine specimens were cultured on MacConkey and Blood agar figure (1), then inoculated at 37°C for 18-24 hours [(7)].

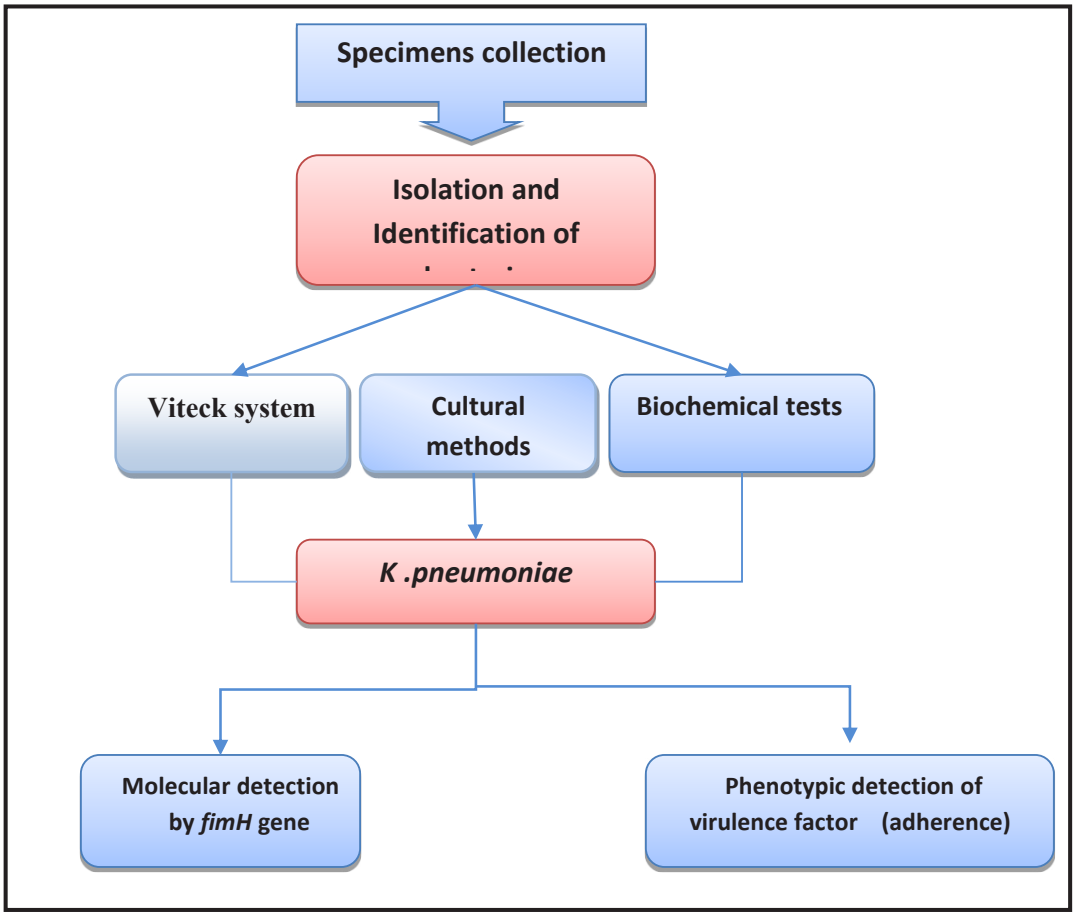


Figure 1: Scheme of the current study

Identification of bacteria

It studied the colonial characteristics such as (shape, volume, color, borders and texture) and examined microscopically after staining with gram-stain [8] and biochemical test for diagnosis of *K. pneumoniae*. Finally, identification was performed with automated VITEK-2 compact system using G-ve ID cards.

Molecular study

Extraction of Genomic DNA

Genomic DNA was extracted by using boiling method DNA was extracted from colonies grown on agar plates by boiling method according to , taking colonies of bacteria grown on MacConkey agar plates were suspended in 300µl of Tris-EDTA Buffer buffer in Eppendorf tube, then vortex and boiling at 100°C for 15 min and immediately freeze at -20°C for 20 min to lyses the organisms and release the DNA, then centrifuged at 8000 xg for 5 min ,supernatant transferred to new Eppendorf tube and stored at -20°C until used.

Polymerase Chain Reaction Protocol

The DNA extract of *K. pneumoniae*. Isolates were subjected to flageller gene genes listed in (Table 1) by using PCR. The protocol was used depending on Promega Biosystem manufacturer's instruction. Single reaction (final reaction volume 20µl as in table (1). All PCR components were assembled in PCR tube and mixed by refrigerated microcentrifuge at 50 rcf for 10 second.

Table 1: Protocol of monplex PCR reaction mixture volumes

Master mix	8µL
DNA template	5µL
Forward primers	1.5µL
Reverse primers	1.5µL
Deionidied water (d d water)	4µ L
Final volume 20 µL	

PCR Thermocycling Conditions

The PCR tubes were placed on the PCR machine and the right PCR cycling program parameters conditions were installed as in table (2).

Agarose Gel Electrophoresis: According to [9].

Table 2: Amplification Conditions of genes were used by PCR reactions.

Gene	Initial denaturation	No. of cycles	Denaturation	Annealing	Extension	Final extension
fimH	94ºC for 4min.	35	94ºC for 30sec.	52.9ºC for 30sec.	72ºC for 50sec.	72ºC for 7min.

Results and Discussion

Prevalence of *K. pneumoniae* specimens

This study was conducted on 40 specimens from urine urinary catheterized patients during the period from October 2018 to March 2019, all these specimens 40 were inoculated on MacConkey and Blood agar medium as at 37°C for 18-24 hours [7].

***K. pneumoniae* identification**

Morphologically characterization

The bacterial isolates obtained from clinical samples were identified initially according to cultural morphology, microscopic characteristics and biochemical tests. From those isolates, the cultural identification of *K. pneumoniae* was depended on the colonial morphology. Since the colonies of *K. pneumoniae* were grown on blood agar appears non-haemolytic smooth white colonies and red colour like shaped and smooth colonies when grown on the MacConkey agar, indicated that *K. pneumoniae* is able to ferment lactose sugar (Figure 4-1).

The results of biochemical tests that recorded in table (3) were considered as a complementary of the initial identification of *K. pneumoniae* isolates. The isolates confirm to general characteristics, isolates were negative for oxidase test . Urease production and Simmon citrate utilization and catalase test positive result. All the result (morphology and cultural) were identical with [10].

Table 3: The Biochemical features of *K. pneumoniae*

Test	Result
Oxidase test	-
Simmons Citrate	+
Urea hydrolysis	+
Catalase test	+

Upon detailed bacteriological investigation based on the morphological, cultural and biochemical tests were 40 isolates as tentatively identified as *K. pneumoniae*.

The final identification was performed with the automated VITEK-2 compact system using GN-ID cards

which contained 47 biochemical tests and one negative control well (Appendix1). The results demonstrate that 40 isolates from urine urinary catheterized patients were confirmed as *K. pneumoniae* with ID message confidence level ranging between very good to excellent (Probability percentage 99).

Virulence factors of *K. pneumoniae*

Adherence Variation

Biofilm forming ability is highly linked to bacteria swarming in, which is represented as an important virulent factor [11, 12]. All of the tested isolates showed (2cm-8cm) swarming (increase in colony diameter) after 24 hrs of incubation. The rates of the migration were measured at 0, 6, 12, 18 and 24 hr. At 0 hr, *K. pneumoniae* isolates appeared with no migration. At 6 hr and 12 hr, *K. pneumoniae* isolates appeared with same rate of migration. After 18-24 hrs, most of the *K. pneumoniae* isolates covered the entire media surface. These results agree with the findings of [12] who reported more than 1.5Cm – 7.7Cm after 24 hrs of incubation.

Molecular Study

Genomic DNA Extraction

DNA Genomic was successfully extracted from *K.pneumoniae* isolates by using boiling method. The concentration and purity of extracted DNA were directly determined by spectrophotometry, extracted DNA purity ranged between (1.8 - 2). Extracted DNA were confirmed and analyzed by gel electrophoresis.

Molecular Detection of *fimH* gene of *K. pneumoniae*

The results showed that *fimH* gene was detected in 14 / 40 of *K. pneumoniae* isolates as in figure (2).

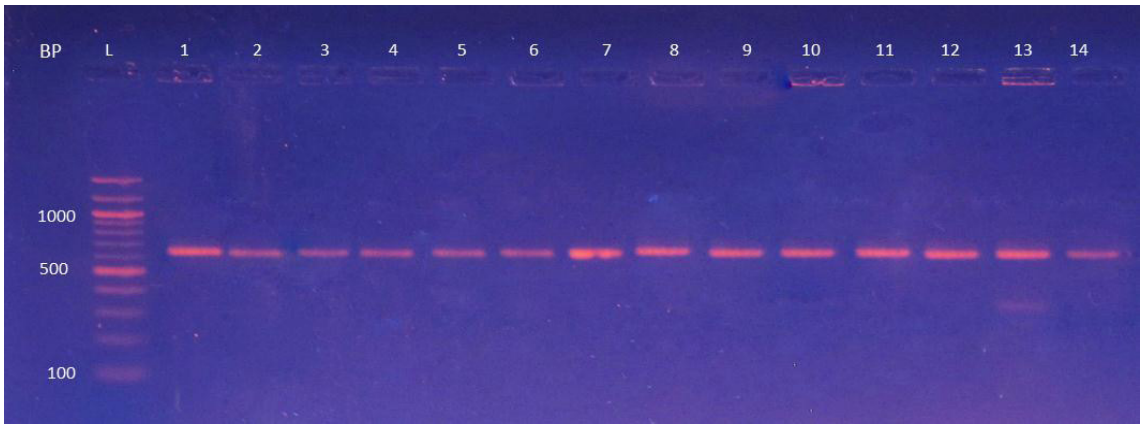


Figure 2: PCR amplicon of *K. pneumoniae fimH* gene. Product size 688bp. Lane (L), DNA marker (100-bp ladder), Lanes (1 to 14) positive results.

Fimbriae are assumed to play critical roles in attachment to epithelial cell surfaces. Binding to specific host receptors, fimbriae mediates the bacterial colonization, host cell signaling. Fimbrial adhesins determine the fate of the bacterial pathogen in the host as well as the progress of the corresponding disease process .Type-1 fimbriae also play an important role in deciding the virulence of the organism. Experiments conducted by Jaroni indicated that a mannose-resistant haemagglutinin was required for the attachment of Klebisella to target cells [13]. The present result assumed important role of fimbriae 1 in attachment to epithelial cell surface (mediates the bacterial colonization) and deciding the virulence of the *K. pneumoniae*. The relationship between mannose – sensitive hemagglutinin (MSHA) or type 1 fimbriae and pathogenisity of bacteria was established from adherence of bacteria in mucous surfaces or epithelial cells of gastric tract [14].

Conclusions

The use of Vitek-2 system, is necessary to confirm precise identification of this pathogen. Clinical isolates of *K. pneumoniae* possess number of virulence factor that associated with Urinary Catheter such as adhesion factors (biofilm). The *fimH* gene that encoded for adhesion factors (biofilm) was found almost in *K. pneumoniae* isolates

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-Funding

References

1- Frank DN, Wilson SS, St Amand AL, Pace NR. Cultureindependent microbiological analysis of Foley urinary catheter biofilms 2009.

2- Spencer, R. C. Predominant pathogens found in the European Prevalence of Infection in Intensive Care Study. Eur. J. Clin. Microbiol. Infect., 1996.

3- Struve, C., and K. A. Krogfelt. Role of capsule in Klebsiella pneumoniae virulence: lack of correlation between in vitro and in vivo studies. FEMS Microbiol.,2003.

4- Gerlach, G. F., S. Clegg, and B. L. Allen. Identification and characterization of the genes encoding the type 3 and type 1 fimbrial adhesins of Klebsiella pneumoniae. J. Bacteriol., 1989.

5- Diancourt, L., V. Passet, J. Verhoef, P. A. Grimont, and S. Brisse. Multilocus sequence typing of Klebsiella pneumoniae nosocomial isolates. J. Clin. Microbiol., 2005.

6- Fouts, D. E., H. L. Tyler, R. T. DeBoy, S. Daugherty, Q. Ren, J. H. Badger, et. al. Complete genome sequence of the N2-fixing broad host range endophyte Klebsiella pneumoniae 342 and virulence predictions verified in mice. PLoS Genet., 2008.

7- Chessbrough, M. District Laboratory Practice in Tropical Countries, Part-2, New York, USA, Cambridge University, 2010, 184- 186.

8- Jawetz, E.; Melnick, J.I. and Adelberg, E.A. Medical Microbiology . (24nd Ed.). Appleton and Lange U.S.A., 2007.

9- Bartlett, J. S. and Stirling D. PCR Protocols: Methods in Molecular and Biology. 2th. Humana Press Inc. Totowa. NJ. 1998.

10- Collee, J. G.; Fraser, A. G.; Marmion B. P. and Simmon A. Pactical Medical Microbiology. 14th Ed. Churchill Living Stone , USA Collins, C.H. and Lyne, P.M.. Microbiological Methods. 14th Ed., Butterworth and Co. (Pubilisher). 1996.

11- Murray, TS.; Ledizet, M. and Kazmierczak, BI. Swarming motility, secretion of type 3 effectors and biofilm formation phenotypes exhibited within a large cohort of Pseudomonas aeruginosa clinical isolates. J Med Microbiol., 2010. 59(5):511–520.

12- Kalaivani Kalai Chelvam1,2.; Lay Ching Chai1,2. and Kwai Lin, Thong1,2. Variations in motility and biofilm formation of Salmonella enterica serovar typhi. Kalai Chelvam. Gut Pathogens, 2014. 6:2.

13- Jaroni, Salmonella/ *Salmonella typhi* Encyclopedia of Food Microbiology (Second Edition), 2014: 349–352.

14- Nowsheen ,H. and S. K, Jain. Characterization of an outer membrane protein of Salmonella enterica serovar typhi that confers protection against typhoid clinical and vaccine immunology, 2008: 1461– 1471.

Chemerin Level as a Marker in Preeclampsia and its Relation to the Disease Severity and Neonatal Outcome

Ali Mohammed Murad, Marwa Jaafar Sadac (M.B.Ch.B)2; Hanan Hazim Mohammed Noori (M.B.Ch.B, C.A.B.O.G, DGO)3

[1]Department of Obstetrics and Gynecology, College of Medicine, Mustansiriya University, Baghdad, Iraq, [2]Ministry of Health, Al-Yarmouk Teaching Hospital, Baghdad, Iraq, [3]Head of Department of Gynecology and Obstetrics, Al-Qaim Hospital, Al-Anbar Province, Iraq

Abstract

Aim of study: To detect serum chemerin level in patient with preeclampsia and evaluate the association between maternal serum chemerin, disease severity and neonatal outcome.

Patients and Method: A case control study included 100 pregnant women with singleton pregnancy, gestational age of 20 weeks or more, normal fetal morphology, and absence of concomitant diseases, who were collected from inpatient during delivery was conducted in the Department of Obstetrics and Gynecology at Al-Yarmouk Teaching Hospital during the period from 1st of June 2018 till end of May 2019. They were divided into three groups (control, mild preeclampsia, and severe preeclampsia). Patients with history of chronic hypertension, diabetes mellitus, cardiovascular disease, neurological disorder, renal impairment, or premature rapture of membrane were excluded from this study. blood sample was taken from all patients and sent for human chemerin assay. After delivery, birthweight of baby, APGAR scores at one and five mints, neonatal intensive care unit and adult intensive care unit admission, and hospitalization time were also noted.

Results: There were no statistically significant differences between the study groups in age, BMI level, and parity. Chemerin level was significantly elevated in patients with severe preeclampsia (435.06 ng/ml) and mild preeclampsia (227.49 ng/ml) than that in non-preeclamptic patients (202.6 ng/ml). It was negatively correlated with each of gestational age, birth weight, Apgar score at one and five minutes. While it was positively correlated with admission's duration. Serum chemerin > 228.5 ng/ml is predictive for diagnosis of preeclampsia and level > 380.9 ng/ml is indicator for severe preeclampsia.

Conclusion: Chemerin may play a role in the pathogenesis of preeclampsia as maternal serum chemerin level was significantly higher in patients with preeclampsia

Keywords: *Preeclampsia, chemerin, APGAR score, birthweight, Iraq*

Introduction

Preeclampsia (PE) is a syndrome that chiefly includes the new onset of hypertension and either proteinuria or signs of other end-organ dysfunction (e.g. hepatic abnormality, pulmonary edema, thrombocytopenia) [(1)]. PE affect between 3% and 5% of all pregnancies and account for more than 60,000 maternal and 500,000 fetal deaths per year worldwide [(2)]. It is one of the most important causes of maternal, perinatal, and fetal morbidity and mortality in the world [(3)]. Deficient spiral artery remodeling, placental ischemia, release of mediators into the maternal circulation, systemic endothelial dysfunction, inflammation, and consequent increased vascular constriction are biological mechanisms that contribute to preeclampsia [(4, 5)]. Additionally, abnormal placentation, imbalance of angiogenesis regulators, and maternal immune

Corresponding Author:
Hanan Hazim Mohammed Noori
E-mail: dr.ammar2013@gmail.com

maladaptation are other possible factors associated with preeclampsia [6]. Moreover, the future risk of vascular and metabolic disease is significantly increased after a preeclamptic pregnancy [7]. However, the pathogenesis of this life-threatening condition remains unclear [8]. The placenta is thought to be a major source of endogenous nitric oxide (NO) during pregnancy. Endothelial NO, which is synthesized by endothelial NO synthase (eNOS), is an important regulator of blood flow and vasomotor tone via its inhibition of smooth muscle contraction. Thus, it is hypothesized that NO–eNOS system abnormalities are associated with the onset of preeclampsia [9, 10]. Chemerin, named also as tazarotene-induced gene protein 2or retinoic acid receptor responder protein 2, is a novel adipocy-tokine that is mainly expressed in adipocytes, liver, placenta, and ovaries [11]. Evidence has been presented that chemerin is linked to facets of the metabolic syndrome in vitro and in vivo [12] hence this adipokine is correlated with insulin resistance and body fat accumulation [13]. Hypertension, coronary disease [14], diabetes mellitus [15], atherosclerosis [16], obesity and metabolic syndrome [17] are also associated with chemerin. Although its specific biological functions are controversial, chemerin may play a role in the pathogenesis in preeclampsia. The aim of this study is to determine whether serum chemerin concentrations are elevated in preeclamptic women and whether serum chemerin levels differ according to severity of preeclampsia and to evaluate the association between maternal serum chemerin and neonatal outcome.

Patients and Method

Study design, setting: This is a case control study that was conducted in the Department of Obstetrics and Gynecology at Al-Yarmouk Teaching Hospital during the period from 1st of June 2018 till end of May 2019.

Study Population and sample size: The study included 100 pregnant women with singleton pregnancy, gestational age of 20 weeks or more, normal fetal morphology, and absence of concomitant diseases, who were collected from inpatient during delivery in labor room. They were divided into three groups:

- Severe Group: Included 33 pregnant women who had diagnosed with severe PE.
- Mild Group: Included 33 pregnant women who had diagnosed with mild PE.
- Control Group: Included 34 pregnant women with uncomplicated pregnancy who were selected after matching for age and gestational age of another one in the other two groups after proof that she was normotensive by history, examination and investigation.

Mild PE is diagnosed when hypertension with two readings (separated by 4-6 hrs. apart) of systolic blood pressure ≥ 140 mmHg and / or diastolic pressure ≥ 90 mmHg. Another characteristic feature of mild PE is the development of protein urea ≥ 300 mg / 24 hrs. [18]. Severe PE can be identified by presence of sustained elevation in blood pressure, systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 110 mmHg, proteinuria of > 2 gm in a 24-hrs urine specimen, serum creatinine of >1.2 mg/dl, platelets of < 100,000/dl, increased lactate dehydrogenase, elevated serum transaminase levels, persistent headache, and oliguria (urinary output<400 ml/ 24 hours). In addition, any patient with cerebral or visual impairment, persistent epigastric pain, pulmonary edema or cyanosis, impaired liver function, or thrombocytopenia (platelet count less than 100,000/ml) was diagnosed with severe PE [3]. Pregnant women with history of chronic hypertension, diabetes, cardiovascular disease, neurological disorders, renal impairment, or with premature rapture of membrane were excluded from this study.

Data collection: All patient told about the nature of the study and verbal consent was taken from them. Information about maternal age, gestational age, parity, gravidity, mode of delivery, previous history of preeclampsia, family history of any previous medical history. Then both group undergo to general examination, vital signs (systolic and diastolic blood pressure), abdominal and obstetric examination, laboratory investigation and sonographic examination. Then, from all study patients, we took 10 ml blood sample which was divided into two equal parts, the first one was sent to our hospital laboratories for CBC, blood group and Rh, blood sugar, b. urea, s. creatinine, SGOT, SGPT, coagulation profile and platelet count. In the other part, serum was separated by centrifugation at 2,500 rpm for 10 min and frozen at -70 °C. The serum chemerin levels were measured by enzyme linked immunosorbent assay according to the manufacturer's instructions. The lowest level of human chemerin that can be detected by this assay is 31.2 ng/ml.

After delivery, birthweight of baby, APGAR scores at one and five mints, neonatal intensive care unit

(NICU) and adult intensive care unit (AICU) admission, and hospitalization time were also noted.

Statistical analysis

The data analyzed using Statistical Package for Social Sciences (SPSS) version 25. They presented as mean, standard deviation and ranges. Categorical data presented by frequencies and percentages. Analysis of Variance (ANOVA) (two tailed) was used to compare the continuous variables accordingly. Pearson's correlation test (r) was used to assess correlation between continuous variables accordingly. Receiver operating characteristic (ROC) curve analysis was used for prediction of chemerin level as diagnostic of preeclampsia. A level of P – value less than 0.05 was considered significant.

Results

In this study, 100 pregnant women were enrolled. The age was ranging from 17 to 40 years with a mean of 25.9 ± 6.13 years. Regarding general characteristics, there were no statistical significant differences ($P \geq 0.05$) between the study groups in age, BMI, and parity. Concerning blood pressure, SBP and DBP were significantly higher in severe group than that in mild and control groups (169.34 versus 148.93 and 123.81 mmHg, P= 0.001; and 107.1 versus 95.9 and 75.53 mmHg, P= 0.001 respectively).

About investigation, AST, ALT, s. urea and chemerin level were significantly higher in severe group than that in mild and control groups (155.06 versus 45.48 and 30.37 U/l, P= 0.001; 126.0 versus 35.36 and 25.71 U/l, P= 0.001; 31.65 versus 25.0 and 25.78 mg/dl, P= 0.001; and 435.06 versus 227.49 and 202.6 ng/ml, P= 0.001 respectively). Mean of platelet count was significantly lower in severe group than that in mild and control groups (101.65 versus 149.21 and 198.09, P= 0.001)

No statistical significant differences ($P \geq 0.05$) between the study groups in WBC and s. creatinine as shown in table (1).

Table 1: Comparison between study groups by general characteristics, blood pressure, and investigation

Variable	Severe Group Mean ± SD	Mild Group Mean ± SD	Control Group Mean ± SD	P - Value
General characteristics				
Maternal age (Year)	24.45 ± 6.3	25.0 ± 5.0	26.23 ± 6.5	0.564
BMI (Kg/m2)	28.43 ± 3.8	26.61 ± 3.8	29.35 ± 4.1	0.093
Parity	1.36 ± 1.7	1.37 ± 1.7	1.35 ± 1.7	0.279
Blood pressure				
SBP (mmHg)	169.34 ± 17.3	148.93 ± 6.2	123.81 ± 10.1	0.001
DBP (mmHg)	107.1 ± 20.1	95.9 ± 5.3	75.53 ± 7.8	0.001
Investigation				
WBC (10 9/l)	12.32 ± 2.8	11.17 ± 3.9	10.62 ± 3.4	0.127
AST (U/l)	155.06 ± 210.9	45.48 ± 7.4	30.37 ± 6.6	0.001
ALT (U/l)	126.0 ± 148.6	35.36 ± 7.5	25.71 ± 6.2	0.001
Urea (mg/dl)	31.65 ± 10.0	25.0 ± 4.2	25.78 ± 5.4	0.001
Creatinine (mg/dl)	0.71 ± 0.23	0.77 ± 0.16	0.81 ± 0.16	0.126
PLT Count (10 9/l)	101.65 ± 19.5	149.21 ± 26.6	198.09 ± 37.2	0.001
Chemerin level (ng/ml)	435.06 ± 55.4	227.49 ± 57.4	202.6 ± 21.1	0.001

Receiver operating characteristic (ROC) curve analysis was constructed for chemerin level as indicator of preeclampsia. As shown in figure (1) and table (2), the cut point of chemerin level was 228.5 ng/ml, so s. chemerin > 228.5 ng/ml is predictive for diagnosis of preeclampsia indicating significant association between higher level of chemerin and diagnosis of preeclampsia.

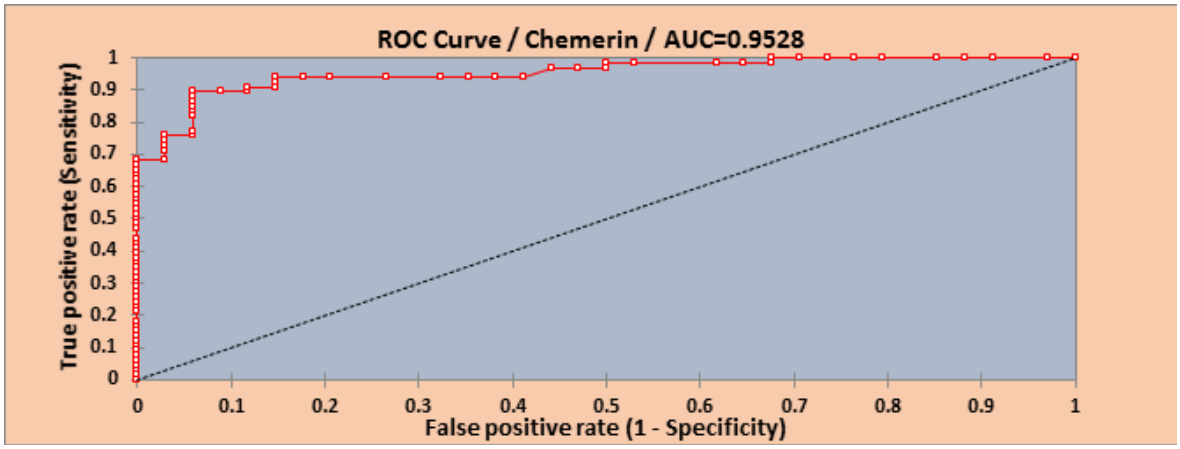


Figure 1: ROC curve for chemerin as a marker of preeclampsia

Table 2: Diagnostic accuracy for test of preeclampsia

	Cut-off value	**Sensitivity**	**Specificity**	**PPV**	**NPV**	Accuracy
Chemerin level (ng/ml)	228.5	89.3%	94.1%	96.7%	82%	91%

ROC curve analysis was constructed again for chemerin level as diagnostic for severity of preeclampsia. As shown in figure (2) and table (3), the cut point of chemerin level was 380.9 ng/ml, so s. chemerin > 380.9 ng/ml is indicator for severe preeclampsia indicating significant association between higher level of chemerin and diagnosis of severe preeclampsia.

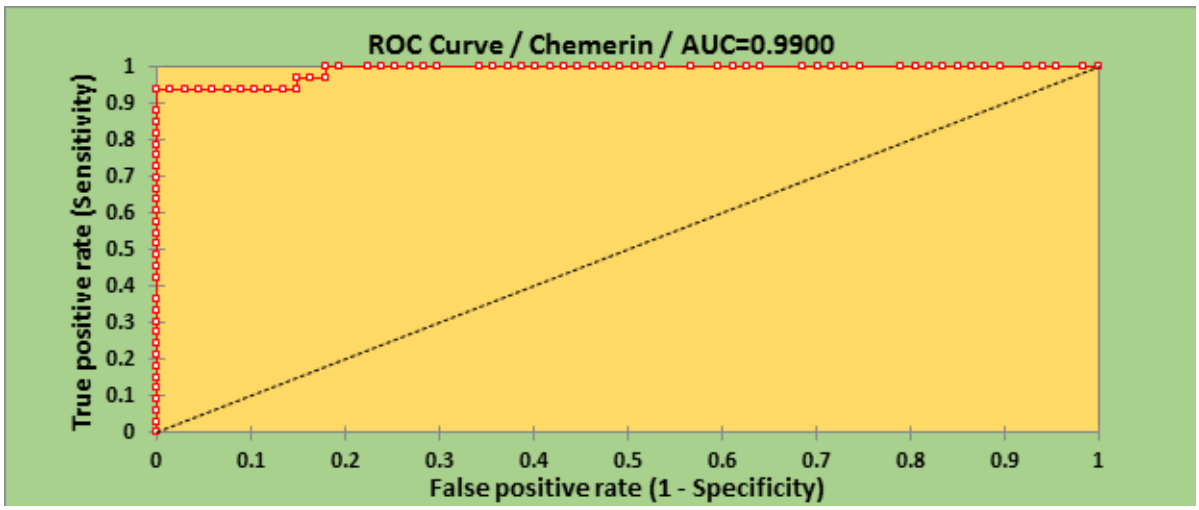


Figure 2: ROC curve for chemerin level as a marker of severe preeclampsia

Table 3: Diagnostic accuracy for test of severe preeclampsia

	Cut-off value	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**
Chemerin Level (ng/ml)	380.9	93.9%	100%	100%	97.1%	98%

Chemerin level was negatively correlated with each of gestational age, birth weight, Apgar score at one and five minutes. While it was positively correlated with admission's duration, and these correlations were demonstrated in table (4).

Table 4: Correlation between level of chemerin and certain obstetric and neonatal outcomes of the study groups

Variable	Chemerin Level (ng/ml)	
	r	P - Value
Gestational age (Weeks)	- 0.711	0.001
Birth Weight	- 0.714	0.001
Apgar Score at 1 Mint	- 0.615	0.001
Apgar Score at 5 Mint	**- 0.709**	**0.001**
Duration of Admission	**0.547**	**0.001**

Discussion

Recently, it has been reported that circulating chemerin concentrations were strongly correlated with the key markers of the metabolic syndrome, including insulin resistance, hyperlipidemia, and high blood pressure. In the current study, we found that maternal serum chemerin level was significantly higher in severe and mild preeclamptic patients compared to healthy pregnant women, and elevated serum chemerin level (>228.5 ng/ml) indicated preeclampsia with 89.3% sensitivity and 94.1% specificity and > 380.9 ng/ml indicated severe preeclampsia with 93.9% sensitivity and 100% specificity. These result was agreed with results conducted by Cetin et al study 2017 [3], Xu QL et al study 2014 [19], Wang L et al study 2015 [8], Duan DM et al study 2012 [20], and Stepan H et al study 2011 [21] when they all showed that serum chemerin was significantly higher in patients with preeclampsia than that of healthy pregnant women. These findings indicate that differential expressions of chemerin may be responsible for pathological changes in patients with preeclampsia. Chemerin was identified in maternal circulation during pregnancy. Placenta releases the major part of chemerin during the gestational period and also it plays a critical role in controlling /contributing to metabolic processes [22, 23]. The signaling pathway between high expression of chemerin and its receptor CMKLR1 is the common pathogenic factor of obesity, diabetes, hypertension and metabolic syndrome. Meanwhile, chemerin is also overexpressed in preeclampsia. It is a matter of further investigation to determine whether the chemerin/CMKLR1 signaling pathway is an internal factor between preeclampsia and obesity, diabetes, hypertension and metabolic syndrome [24]

In this study, chemerin level was negatively correlated with each of gestational age, birth weight, Apgar score at one and five minutes, while it was positively correlated with admission's duration. This is similar to studies conducted by Cetin et al 2017 [3], and by Duan DM et al 2012 [20] Additionally, preeclampsia can lead to higher frequency of induced labor, neonatal respiratory difficulties, and increased frequency admission to neonatal intensive care unit [25]. In conclusion, maternal serum chemerin level is significantly increased in preeclampsia, especially in severe preeclampsia. Larger studies and work are needed to better determine the mechanisms by which serum chemerin is increased in preeclampsia.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Girsberger M, Muff C, Hösli I, Dickenmann MJ. Short term sequelae of preeclampsia: a single center

cohort study. BMC Pregnancy and Childbirth. 2018;18(1):177.

2. Peres GM, Mariana M, Cairrão E. Pre-Eclampsia and Eclampsia: An Update on the Pharmacological Treatment Applied in Portugal. J Cardiovasc Dev Dis. 2018;5(1):3.
3. Cetin O, Kurdoglu Z, Kurdoglu M, Sahin HG. Chemerin level in pregnancies complicated by preeclampsia and its relation with disease severity and neonatal outcomes. Journal of Obstetrics and Gynaecology. 2017;37(2):195-9.
4. Adali E, Kurdoglu Z, Kurdoglu M, Kamaci M, Kolusari A, Yildizhan R. Metastin levels in pregnancies complicated by pre-eclampsia and their relation with disease severity. The Journal of Maternal-Fetal & Neonatal Medicine. 2012;25(12):2671-5.
5. Kurdoglu Z, Ozkol H, Kurdoglu M, Kamaci M. Evaluation of the relationship between adenosine deaminase, myeloperoxidase, cholinesterase, preeclampsia severity, and neonatal outcomes. Clinical and experimental hypertension. 2012;34(7):493-7.
6. Pennington KA, Schlitt JM, Jackson DL, Schulz LC, Schust DJ. Preeclampsia: multiple approaches for a multifactorial disease. Disease models & mechanisms. 2012;5(1):9-18.
7. Hedderson MM, Ferrara A. High blood pressure before and during early pregnancy is associated with an increased risk of gestational diabetes mellitus. Diabetes care. 2008;31(12):2362-7.
8. Wang L, Yang T, Ding Y, Zhong Y, Yu L, Peng M. Chemerin plays a protective role by regulating human umbilical vein endothelial cell-induced nitric oxide signaling in preeclampsia. Endocrine. 2015;48(1):299-308.
9. Dai B, Liu T, Zhang B, Zhang X, Wang Z. The polymorphism for endothelial nitric oxide synthase gene, the level of nitric oxide and the risk for pre-eclampsia: a meta-analysis. Gene. 2013;519(1):187-93.
10. Drews K, Kraśnik W, Kurzawińska G, Barlik M, Perlik M, Seremak-Mrozikiewicz A. Genetic variants of endothelial nitric synthase in gestational hypertension and preeclampsia. Ginekologia polska. 2012;83(9).
11. Rourke J, Dranse H, Sinal C. Towards an integrative approach to understanding the role of chemerin in human health and disease. Obesity Reviews. 2013;14(3):245-62.
12. Bozaoglu K, Bolton K, McMillan J, Zimmet P, Jowett J, Collier G, et al. Chemerin is a novel adipokine associated with obesity and metabolic syndrome. Endocrinology. 2007;148(10):4687-94.
13. Fatima SS, Bozaoglu K, Rehman R, Alam F, Memon AS. Elevated chemerin levels in Pakistani men: an interrelation with metabolic syndrome phenotypes. PloS one. 2013;8(2):e57113.
14. Yan Q, Zhang Y, Hong J, Gu W, Dai M, Shi J, et al. The association of serum chemerin level with risk of coronary artery disease in Chinese adults. Endocrine. 2012;41(2):281-8.
15. Roman AA, Parlee SD, Sinal CJ. Chemerin: a potential endocrine link between obesity and type 2 diabetes. Endocrine. 2012;42(2):243-51.
16. Hah Y-J, Kim N-K, Kim M-K, Kim H-S, Hur S-H, Yoon H-J, et al. Relationship between chemerin levels and cardiometabolic parameters and degree of coronary stenosis in Korean patients with coronary artery disease. Diabetes & metabolism journal. 2011;35(3):248-54.
17. Ress C, Tschoner A, Engl J, Klaus A, Tilg H, Ebenbichler C, et al. Effect of bariatric surgery on circulating chemerin levels. European journal of clinical investigation. 2010;40(3):277-80.
18. Mol BW, Roberts CT, Thangaratinam S, Magee LA, De Groot CJ, Hofmeyr GJ. Pre-eclampsia. The Lancet. 2016;387(10022):999-1011.
19. Xu Q-L, Zhu M, Jin Y, Wang N, Xu H-X, Quan L-M, et al. The predictive value of the first-trimester maternal serum chemerin level for pre-eclampsia. Peptides. 2014;62:150-4.
20. Duan D-M, Niu J-M, Lei Q, Lin X-H, Chen X. Serum levels of the adipokine chemerin in preeclampsia. Journal of perinatal medicine. 2012;40(2):121-7.
21. Stepan H, Philipp A, Roth I, Kralisch S, Jank A, Schaarschmidt W, et al. Serum levels of the adipokine chemerin are increased in preeclampsia during and 6 months after pregnancy. Regulatory peptides. 2011;168(1-3):69-72.
22. Garces M, Sanchez E, Acosta B, Angel E, Ruiz A, Rubio-Romero J, et al. Expression and regulation of chemerin during rat pregnancy. Placenta. 2012;33(5):373-8.
23. Pfau D, Stepan H, Kratzsch J, Verlohren M, Verlohren H-J, Drynda K, et al. Circulating

levels of the adipokine chemerin in gestational diabetes mellitus. Hormone research in paediatrics. 2010;74(1):56-61.

24. ZHANG Q-Q, FAN X-J, ZHANG J. The role of adipokine chemerin in pregnancy complications. Progress in Biochemistry and Biophysics. 2015;42(10):962-8.

25. Roberts JM, Cunningham FG, Lindheimer MD. Chesley's Hypertensive Disorders in Pregnancy: Academic Press; 2009.

Production, Analysis and Optimization of Inulin Produced from *Pseudomonas fluorescens*

Alyaa Razooqi Hussein[1], Alaa Raheem Kadhim[2]

[1]Assist. Prof. Dr., [2]Lecturer. Dr. Department of Biology, College of Science, University of Baghdad, Baghdad-Iraq

Abstract

Inulin is a natural polysaccharide produced from organisms and microorganism. Inulin is a type of fructan that α-amylase cannot digest it and cannot hydrolysis by hydrolytic enzymes. It was used to get non-fat fermented milk and it's a type of prebiotics that induce growth and activity of probiotics bacteria, thus improving the health. *Pseudomonas fluorescens* have the capacity to produce inulin when grown in media supplied with sucrose. Twenty-two of bacterial isolates were belong to *Pseudomonas fluorescens* depending on structural features, microscopic checking, biochemical analysis and flourescent pigments that produced on King B medium. Ten isolates of *P. fluorescens* had strongly degree of mucous growth on solid production medium, the gummy and mucous manifestation on agar medium containing sucrose were give rise to inulin production. Inulin produced from *P. fluorescens* was analyzed by Fourier Transform Infrared Spectroscopy to detect functional groups which it was (C-O, CH, OH and C=O) and by Thin Layer Chromatography to determine its components of monosaccharide. Inulin distinguished as dark spot on white background and the Rf of it was (0.58). The best product of inulin were in production salt agar medium containing 20% sucrose, pH =7, Temperature =37^{0}C without nitrogen sources and the inoculum size 1%, it was (3.2 gm⁄100 ml). The effects of bacterial inulin on the growth of *Saccharomyces cerevisiae* was studied by culturing it in medium supplemented with 3% of bacterial inulin at 30°C for 48 hrs. The results showed there was no remarkable effect of inulin on *Saccharomyces cerevisiae* growth in comparison to control.

Keywords: *Inulin, Fructan, Levan, Pseudomonas fluorescens, Saccharomyces cerevisiae, Probiotic, Prebiotics, Biochemical test.*

Introduction

Inulin is a natural polysaccharide produced from organisms and microorganism [1, 2]. It is a type of fructan that α-amylase cannot digest it and cannot hydrolysis by hydrolytic enzymes [3,4]. Inulin have protective effect on the survival and activity of lactic acid bacteria when storage and use it as final product [5,6], and it was stimulated the growth of probiotic bacteria thus improving the health [7].In food technology inulin use to improve body mouthful, as stabilizers, fat replacers, and flavour enhacers [8,9]**.** *Pseudomonas fluorescens* capable for adaptation at different environments by extracellular substances [10]. Living micro-organisms and its products are widely used for therapeutic purposes, *Saccharomyces cerevisiae* also possess some medicinal effciency, and the benefcial properties of it are well documented [11,12].

At present day many pharmaceutical preparations with microorganisms products are commercially available [13, 14].

The aim of this study was to seek the capacity of local isolate of *Pseudomonas fluorescens* to produce inulin in different conditions and the effect of inulin on *Saccharomyces cerevisiae* growth.

Materials and Method

Samples collection

Thirty-five of different food samples were collected from different local markets in Baghdad governorate in sterilized utensil and imparted to the laboratory until using.

Sorting and Identity of bacteria

Half-gram were taken from specimen and 4.5 ml of sterilized peptone water were added, next dilutions were done, MacConkey agar was prepared and inoculated with 100 µl from the adequate dilution (1×10^7), incubated at 37°C for 24 hrs. Fluorescing colonies were taken

and streaked again on the same agar medium several times till a pure culture was obtained. Bacterial isolates were identified by using selective medium (King B medium), structural features, microscopic checking and biochemical analysis [15].

Checking of Inulin generating isolates

Purified bacterial isolates were activated in Brain Heart Infusion broth (BHI), after incubation periods (0.1 ml) of culture suspension was streaking on production medium (3gm KH2PO4, 3gm K2HPO4, 0.5 gm MgSO4.7H2O, 20% w/v of sucrose and 2% agar- agar), incubated at 37^0C for 24hrs. Mucoid consistence of bacterial colonies gave marked of inulin production.

Quantitative checking in liquid medium

The highly mucous isolates were selected, 10 ml of (BHI) broth were prepared and inoculated with bacterial isolates then incubated for 18hrs at 37^0C, after incubation periods 100 ml of mineral broth (supplied with 20% of sucrose) was cultivated with 1ml of isolates, incubated for 24hrs, 37^0C. Centrifuge were using (6000rpm, 30 minutes) for extracting inulin by mixing the cell free supernatant with ethanol at rate (1:4) and allowed to stand overnight, the aqueous layer was removed and the layer of inulin was collected in sterilized petri-dish and dried at 60^0C.**[16].**

Diagnosis of Inulin:

a) **Fourier Transform Infrared Spectroscopy (FTIR):** Inulin dried weight was analyzed by using the crystal of potassium bromide (KBr) at rate 1:10 (w/w) [17]

b) **Thin Layer Chromatography (TLC):**This method was done according to (Shida *et al*.(2002) [18] as following:

1. Inulin was dissolved (0.01gm) in 1N HCL and incubated at 70^0C for 3 hrs.

2. About 10 µl of this suspension was taken and spotted plentiful of time away from the below end of TLC plate.

3. Sucrose, Fructose and Glucose solutions were destined and spotted in the same manner and they used as marker.

4. The plate of TLC placed in a closed jar containing separation system (butanol: propanol: D.W.: acetic acid at proportion of (7:5:4:2,v:v:v:v), until spread through the plate at 15 cm.

5. TLC taken and drying up.

6. Dried TLC spirt with TLC diagnosis solution (ethanol:H_2SO_4 at rate 9:1, v:v.), put at (90^0C, 10 minutes) [19].

7. Inulin demonstration as dark spot and the space of it were determination.

8. Relative flow (Rf) estimation as the following:

Distance of the sample mobilized across the plate / Distance of the solvent [20].

Influence of some factors on inulin product

1. **Carbon**

A. Production broth (supplied with 20% of glucose and lactose) were prepared**,** inoculated with bacterial growth culture**,** incubated for 24hrs, 37^0C.

B. Extraction of inulin and determination of dry weight.

C. Comparison with inulin dry weight which extracted from mineral broth containing 20% sucrose.

2. **Nitrogen**

A. Production broth with best carbon source were prepared with addition of 1% (yeast extract and peptone), inoculated with bacterial growth culture**,** incubated for 24hrs, 37^0C.

B. Extraction the product and determination the weight.

3. **pHs**

A. Production broth with best carbon source prepared at pHs (5, 6, 7, 8, 9 and 10), inoculated with bacterial growth culture**,** incubated for 24hrs, 37^0C.

B. Extraction the product and determination the weight.

4. **Temperature**

A. Production broth with best (carbon source and pH) prepared**,** inoculated with bacterial growth culture**,** incubated for 24hrs, at (37, 45 and 50^0C).

B. Extraction the product and determination the

weight.

The effect of inulin on *Saccharomyces cerevisiae* growth

In order to investigate *Saccharomyces cerevisiae* growth in existing inulin, *S.cerevisiae* was cultivated in potato dextrose broth, incubated at 30°C for 48hrs then centrifugation for 15 min with 2500×g at 4ºC. The precipitate was taken and washed by (PBS) (0.1 M phosphate buffer pH 7.4, 0.9% saline) and re suspended in PBS.

The suspension was cultured in medium supplied with 3% inulin, incubated at 30°C for 48hrs. The turbidity of cultured medium was measured at 600 nm for up to 48 hrs and compared with control cultured medium free from inulin [21].

Results and Discussion

Sorting and Identity of *Pseudomonas*:

Thirty-five of different food samples were collected from different local markets in Baghdad governorate. Twenty-tow of this isolates were diagnosed as *Pseudomonas* according to structural features and microscopic checking [17]. Flourescent producing on King B medium and Microscopic examination showed Gram-negative bacilli, non spore former bacteria and biochemical analysis showed (urease+, oxidase +, Gelatine hydrolysis +, Catalase +, Starch hydrolysis -) these results showed that they were identified as strains of *Pseudomonas fluorescens* [22].

Checking of Inulin generating isolates

Pseudomonas fluorescens streaking on production medium; for checking their capability to generate mucus manifestation as marking for Inulin production.

Inulin production was varied from species to species. The gummy and mucous manifestation is coming from manufacturing of polysaccharide [23].

Quantitative checking in liquid medium

Ten isolates of *P. fluorescens* with strongly degree of mucous growth had selected for checking their capability to generate inulin in broth medium that supplied with 20% of sucrose.

The highly mucous appearance of these isolates were taken for creation of inulin in mineral salt broth and the highly product was 3.2 gm/100 ml.

Diagnosis of Inulin:

a) Fourier Transform Infrared Spectroscopy (FTIR): Inulin generated by *P. fluorescens* was analyzed by FTIR spectroscopy to detect the effective structure of inulin.

The results showed the presence of C-O stretching group in 1122.49 cm^{-1} , bending group CH,OH in 1336.58 cm^{-1} and 1434.23 cm^{-1}, stretching C=O in1649.02cm^{-1}, stretching CH in 2891.10 cm^{-1} and 2931.60 cm^{-1}, stretching OH in 3367.48 cm^{-1} and 3431.13 cm^{-1}as showed in figure (1). All these groups (C-O, CH, OH and C=O) are functional groups found in carbohydrates [17].

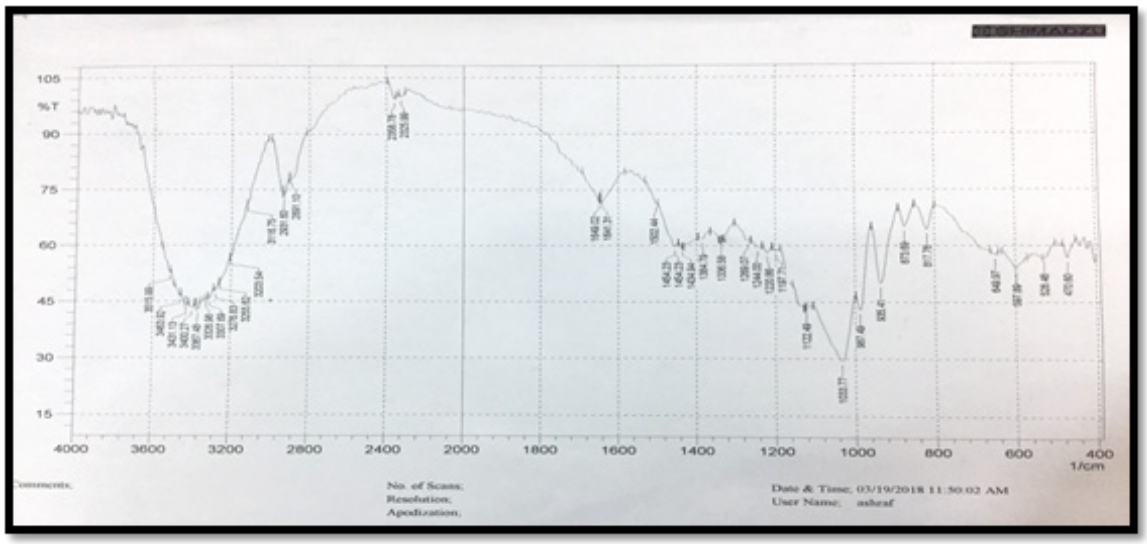


Figure1: FT-IR for inulin production from *P. fluorescens*

Kazim AR. (2015) [17] reported that polysaccharide extracted from *Pseudomonas* does not contain lipids or nucleic acid in structures.

b) Thin Layer Chromatography (TLC): Inulin production from *P. fluorescens* was analyzed by TLC chromatography to determine its components of monosaccharide.

Inulin extracted from *P. fluorescens* was hydrolyzed with HCL before application on TLC and standard sugars (glucose, fructose and sucrose) were prepared and used as marker.

These sugars distinguished as dark spot on TLC plate when using TLC diagnosis solution (ethanol:H_2SO_4 at proportion of 9:1, v:v.) as showed in figure (2).

Rf of these sugars (inulin, fructose, sucrose and glucose) were estimated according to Ghosh, S. and Chandra, A. (1980) [20] and the results were (0.58, 0.59, 0.57 and 0.55) respectively.

Rf for the same compound differed according to (separation system, diagnosis solution and type of solvent) [24].

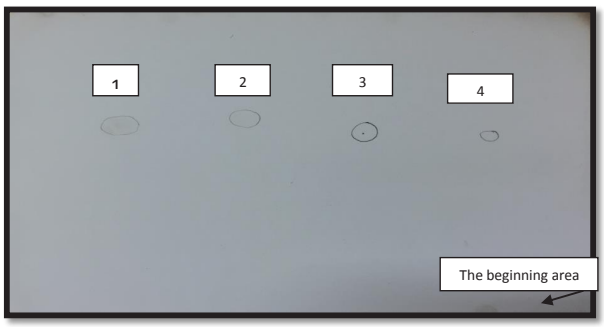


Figure 2: TLC for Inulin production from *P. fluorescens*: Note: (1) inulin, (2)fructose, (3)sucrose and (4)glucose.

Influence of some factors on inulin product

1. **Influence of carbon**

Inulin product may be Influence by the (type, kind and concentration) of carbon substance.

For investigation the influence of carbon, production medium supplied with 20% of glucose, lactose and sucrose inoculated with *Pseudomonas flourescens* and incubated for 24hrs, 37^0C.

From study of the results, the best product of inulin from *Pseudomonas flourescens* was obtained from production medium supplied with sucrose (3.2 gm/100 ml) and the minimum productivity was in existence of glucose (0.2gm/100 ml) as shown in figure**(3).**

The amount of inulin was varied and the highest inulin production was produced when *Pseudomonas flourescens* was cultivated in medium containing 20% of sucrose.

Microorganisms catabolized polysaccharide such as sucrose by levansucrase enzyme which degradation of sucrose [15].

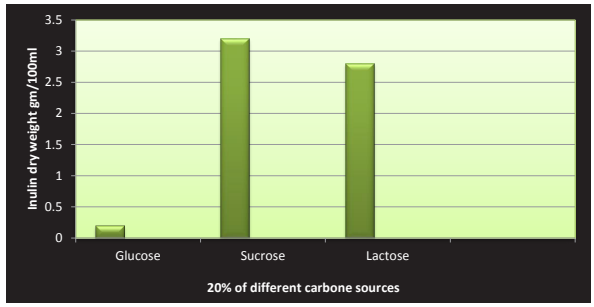


Figure 3: Influence of carbon sources on the productivity of inulin from *Pseudomonas flourescens*

2. **Influence of nitrogen**

By studying the results showed that there was reducing in productivity of inulin when supplying production medium with 1% of yeast extract and peptone.

The productivity decrease from (3.2 gm/100ml) to (2.1 gm/100) when supplied production medium with yeast extract and to (1.8 gm/100ml) in the present of peptone in medium as shown in figure (5).

Microorganisms required nitrogen to complete the metabolic pathway, also nitrogen enhances and increase the microorganism growth; these increasing in growth may be decreased the production of inulin.

The effect of yeast extract in production medium was studied previously, rising concentration of yeast extract responsible for an increase in the *Zymomonas mobilis* and reduction the productivity of inulin, yeast extract also improves and enhancement of substrate consumption, increasing in consumption of substrate verified only a small part of consumed sugar and converted it to Inulin [25].

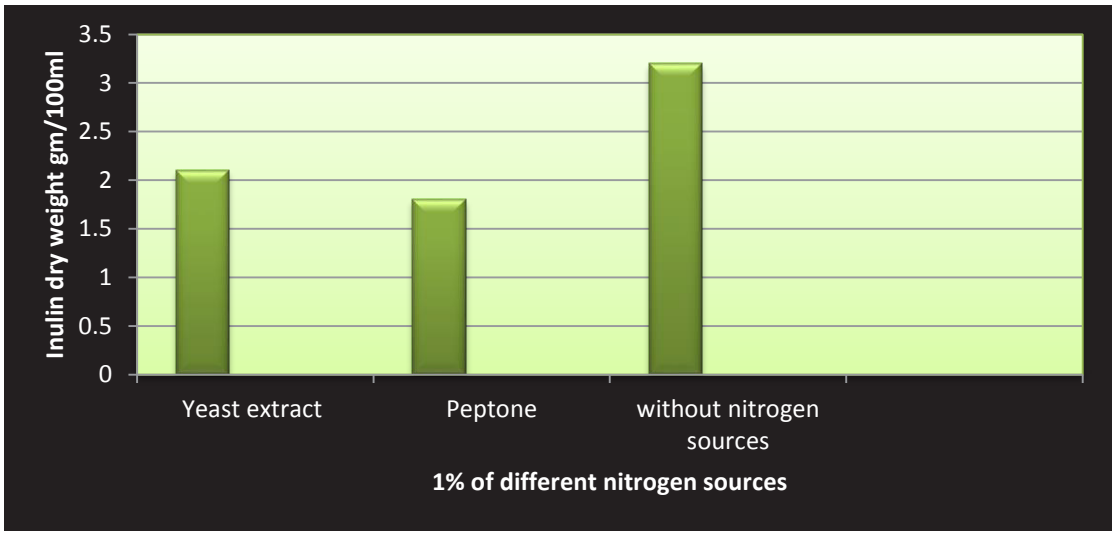


Figure 4: Influence of carbon sources on the productivity of inulin from *Pseudomonas flourescens*

3. Influence of pHs

From the results revealed; inulin productivity was varied with pH of production medium, the productivity decrease in pHs less than 7 and more than 7 as showed in figure (5).

Inulin is chemically stable in a natural and alkaline environment and its stability decreases in an acidic environment [25].

4. Influence of temperature

It was revealed that the best productivity of inulin at 37°C (3.2 gm/100ml) and the productivity was decrease at temperature above than 37°C, it was 2.1gm/100ml at 45°C and 0.1gm/100ml, figure (6).

From the result, the best temperature for the productivity was 37°C and at this temperature enzyme responsible for inulin may be synthesis.

Fructosyltransferase enzymes (FTFs) is bacterial enzymes responsible for synthesis of inulin and frctun from sucrose in three reactions [25].

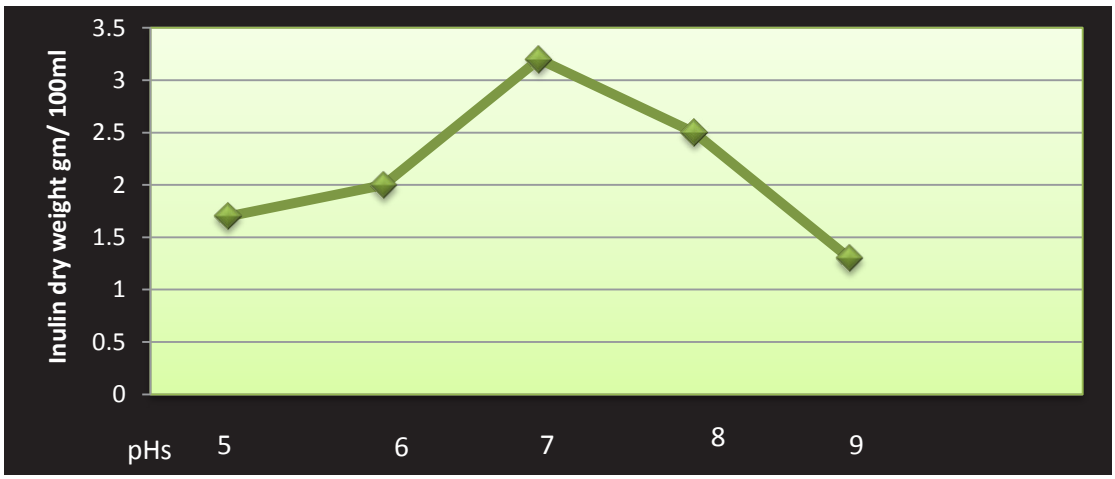


Figure 5: Influence of pHs on the productivity of inulin from *Pseudomonas flourescens*

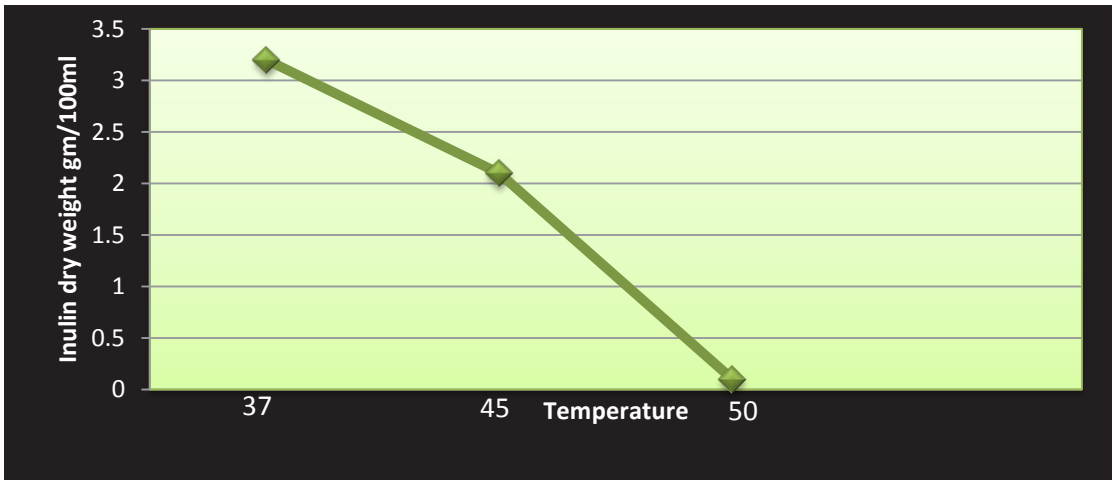


Figure 6: Influence of temperature on the productivity of inulin from *Pseudomonas flourescens*

Fungal Growth study

In order to investigate *Saccharomyces cerevisiae* growth in existing inulin, *S.cerevisiae* suspension was cultured in medium supplied with 3% inulin, incubated at 30°C for 48hrs. The turbidity of cultured medium was measured at 600 nm for up to 48 hrs and compared with control cultured medium free from inulin.

From the results of OD,(OD of control (2.8278), OD of test medium supplied with 3% inulin was 3.0158) there was no remarkable effect of inulin on *S.cerevisiae* growth when compared with the growth of control.

S.cerevisiae had not enzymes responsible for degradation of inulin and used it as carbon sources.

Inulinases hydrolysis of inulin to glucose and fructose; already these molecules can be used as carbon sources to produce many beneficial products for microorganisms.

Conclusion

These studied seeking for the capacity of inulin production from local isolate of *Pseudomonas fluorescens*. The results showed no remarkable effectiveness of inulin on the proliferation of *Saccharomyces cerevisiae* compared with control.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Barclay T, Ginic-Markovic M, Cooper P, Petrovsky N. Inulin: Aversatile polysaccharide with multipharmaceutical and food chemical uses. Journal of Excipients and Food Chemicals. 2010; 1: 27-50.
2. Glibowski P, Bukowska A. The effect of pH, temperature and heating time on inulin chemical stability. ActaScientiarum Polonorum Technologia Alimentaria. 2011; 10: 189-196.
3. Villegas B, Costell E. Flow behaviour of inulin–milk beverages. Influence of inulin average chain length and of milk fat content. International Dairy Journal. 2007; 17: 776-781.Chaillou S.
4. Oliveira RPS, Perego P, Oliveira M N, Converti A. Growth and survival of mixed probioticsin non fat fermented milk: the effect of inulin. Chemical Engineering Transactions. 2011; 24: 457-462.
5. Mattila-Sandholm T, Myllarinen P, Crittenden R, Mogensen G, Fonden R, Saarela M. Technological challenges for future probiotic foods. International Dairy Journal. 2002; 12: 173-182.
6. Donkor ON, Nilmini SLI, Stolic P, Vasiljevic T, Shah NP. Survival and activity of selected probiotic organisms in set-type yoghurt during cold storage. International Dairy Journal. 2007; 17: 657–665.
7. Losada MA, Olleros T. Towards a healthier diet for

the colon: The influence of fructo oligosaccharides and lactobacilli on intestinal health. Nutr. Res. 2002; 22: 71–84.

8. Bodner JM, Sieg J. Fiber. In R.Tarté (Ed.), Ingredients in meat products: properties, functionality and applications. Springer Publishing. USA. 2009.
9. Shoaib M, Shehzad A, Omar M, Rakha A, Raza H, Sharif HR, Shakeel A, Ansari A, Niazi S. Inulin: properties, health benefits and food applications. Carbohydrate Polymers. 2016; 147: 444–454.
10. Moore ERB, Tindall BJ, Dos Santos VAPM, Pieper DH, Ramos JL, Palleron NJ. Non medical: *Pseudomonas. 2006; 646–703 In* Dworkin M, Falkow S, Rosenberg E, Schleifer K H, Stackebrandt E, editors. (ed), Prokaryotes: a handbook on the biology of bacteria. Springer. New York. NY.
11. Pothoulakis C, Kelly CP, Joshi MA. *Saccharomyces boulardii* inhibits *Clostridium difficile* Toxin A binding and enterotoxicity in rat ileum. Gastroenterology. 1993; 104: 1108-1115.
12. Rodrigues ACP, Nardi RM, Bambirra EA, Vieira EC, and Nicoli JR. Effect of *Saccharomyces boulardii* against experimental oral infection with *Salmonella* typhimurium and *Shigella flexneri* in conventional and gnotobiotic mice. Journal of Applied Bacteriology. 1996; 81: 251±256.
13. Gibson GR. Dietary modulation of the human gut microflora using prebiotics. British Journal of Nutrition. 1998; 80: Suppl. S209±S212.
14. Fooks LJ, Fuller R, Gibson GR. Prebiotics, probiotics and human gut microbiology. International Dairy Journal. 1999; 9: 53-61.
15. Alaa RK, Alyaa RHAl, Hala MR. Study the effect of levan produced from *Pseudomonas putida* on phagocytic activity. Current Research in Microbiology and Biotechnology. 2017; 5 (5): 1258-1265.
16. Minj J, Vij S. Effect of Prebiotic Inulin on the Fermentation and Growth Kinetics Pattern of Probiotic Yoghurt Bacteria. International Journal of Current Microbiology and Applied Sciences. 2017; 6 (12): 1755-1768.
17. Kazim AR. Production, optimization, and characterization of cellulose produced from *Pseudomonas* spp. World J Exp Biosci. 2015; 3: 89-93.
18. Shida T, Mukaijo K, Ishikawa S, Yamamota H, Sekiguchi J. Production of long chain levan by a sac C insertional mutant from *Bacillus subtilus* 327 UH . Biosci. Biotechnol. Biochem. 2002; 66(7): 1555-1558.
19. Kojima I, Saito T, Izuka M, Minamiura N, Ono S. Characterization of levan produced by *Serratia sp*. Journal of Fermentation and Bioengineering. 1993; 75(1):9-12.
20. Ghosh S, Chandra A. Production of thermostable amylase by *B. apiaries* CBML 152. In: Advancesin Biotechnology (ed. Moo-young). 1980; 313-321. Pergarmon Press. New York. USA.
21. Fatema AG, Sanaa BA. Optimum Condition of Inulinase Production from *Bacillus cereus* (Be9). Iraqi Journal of Science. 2015; 56(2B): 1315-1323.
22. Bikram N, Sabin B, Jiban S. Identification of *Pseudomonas fluorescens* using different biochemical. International Journal of Applied Biology. 2018; 2(2): 27-32.
23. Han Y, Clarke M A. Production of fructan polyfructose polymers using *Bacillus polymyxa*. United StatesPatent. 1996; 5547863.
24. Shabrukova NV, Shestakova LM, Zainetdinova DR, Gamajurova VS. Research of acid hydrolyses of Chttin-Glucan and Chitosan-Glucan complexes. Chemistry and Computational Simulation. Butlerov Communications. 2002; 2(8):57-60.
25. Glibowski P, Bukowska A. The effect of pH, temperature and heating time on inulin chemical stability. ActaScientiarum Polonorum Technologia Alimentaria. 2011; 10:189-196.

Epidemiology of Hepatitis B and C in Al-Muthanna Province

Adian Abd Alrazak Dakl[1], Wafaa Ayad Alnuaimy[1]

[1]Department of Biology , Science Collage, University of Al-muthanna, Iraq

Abstract

This study systematically reviewed and synthesized available records of hepatitis B and C prevalence in Al-Muthanna province through the last five years from early 2014 till the end of 2018 in Women and Children Teaching Hospital at Al-Muthanna province. The study recorded high prevalence of hepatitis virus at this region, most of the patient were females and HCV was the most prevalent between them, the year of 2016 recorded the highest infection rate. **Conclusion:** High rate of Hepatitis C virus infection among of thalassemia patients

Keywords: *hepatitis , blood donor, thalassemia*

Introduction

Hepatitis is an inflammation of the liver, most commonly caused by a viral infection. There are 5 main hepatitis viruses, referred to as types A, B, C, D and E. These five types are of greatest concern because of the burden of illness and death they cause and the potential for outbreaks and epidemic spread. In particular, types B and C lead to chronic disease in hundreds of millions of people and, together, are the most common cause of liver cirrhosis and cancer. Hepatitis A and E are typically caused by ingestion of contaminated food or water. Hepatitis B, C and D usually occur as a result of parental contact with infected body fluids. Common modes of transmission for these viruses include receipt of contaminated blood or blood products, invasive medical procedures using contaminated equipment and for hepatitis B transmission from mother to baby at birth, from family member to child, and also by sexual contact [(1)].

The most common diseases that are transmitted through blood are hepatitis B and hepatitis C viruses [(2)] . Infections with hepatitis B virus (HBV) and hepatitis C virus (HCV) are a worldwide public health problem. In Iraq, viral hepatitis prevention and control program was started during early seventies [(3)].

The transmission of HCV is primarily through exposure to infected blood. Risks for transmission include blood transfusion before 1992, intravenous drug use, high risk sexual activity, solid organ transplantation from an infected donor, occupational exposure, hemodialysis, household exposure, birth to an infected mother, and intranasal cocaine use [(4)].

Review

Hepatitis B and C

Hepatitis B virus is a member of the Hepadnavirus family [(5)]. The virus particle, called Dane particle [(6)] (virion), consists of an outer lipid envelope and an icosahedral nucleocapsid core composed of protein. The nucleocapsid encloses the viral DNA and a DNA polymerase that has reverse transcriptase activity similar to retroviruses [(7)].

Viral infection by hepatitis B virus (HBV) causes many hepatocyte changes due to the direct action of a protein encoded by the virus, HBx, and to indirect changes due to a large increase in intracellular reactive oxygen species (ROS) after infection. HBx appears to dysregulate a number of cellular pathways. HBx causes dysregulation in part by binding to genomic DNA, changing expression patterns of miRNAs, affecting histone methyl transferases, binding to SIRT1 protein to activate transcription, and cooperating with histone methylases and demethylases to change cell expression patterns [(8)].

HBx is partly responsible for the approximate 10,000-fold increase in intracellular ROS upon chronic HBV infection [(9)]. Increased ROS can be caused, in part, by localization of HBx to the mitochondria where HBx decreases the mitochondrial membrane potential, in addition, another HBV protein, HBsAg, also increases

ROS through interactions with the endoplasmic reticulum [10].

Hepatitis C (originally "non-A non-B hepatitis") is caused by hepatitis C virus (HCV), an RNA virus of the family Flaviviridae. HCV can be transmitted through contact with blood (including through sexual contact if the two parties' blood is mixed) and can also cross the placenta. Hepatitis C usually leads to chronic hepatitis, culminating in cirrhosis in some people. It usually remains asymptomatic for decades. Patients with hepatitis C are susceptible to severe hepatitis if they contract either hepatitis A or B, so all persons with hepatitis C should be immunized against hepatitis A and hepatitis B if they are not already immune, and avoid alcohol. HCV viral levels can be reduced to undetectable levels by a combination of interferon and the antiviral drug ribavirin. The genotype of the virus is the primary determinant of the rate of response to this treatment regimen, with genotype 1 being the most resistant. Hepatitis C is the most common chronic blood-borne infection in the United States [11].

Material and Method

The results of the last five years were collected from (Al-Muthanna Women and Children Teaching Hospital) and organized in tables and charts based on gender, age, year of injury, accompanying diseases and residential areas.

Results and Discussion

Thirty three cases were identified through the last five years 13 (39%) of them were infected with HBV and 20 (61%) were infected with HCV, this indicates that the type C was more prevalent during these years. 85% of infection were females as the results were collected from delivery hospital.

Table (1): Distribution of HBV and HCV

Item		NO.	%
IgG ELISA	HBV	13	39%
	HCV	20	61%
Age	≥10	4	12.1%
	11-20	8	24.2%
	21-30	13	39.3%
	31-40	8	24.2%
Genus	Male	5	15%
	Female	28	85%
Year of infection	2014	11	33.3%
	2015	0	0%
	2016	12	36.3%
	2017	6	18.1%
	2018	4	12.1%
Region	Rural	17	52%
	Urban	16	48%
Accompanying disease	Thalassemia	3	9%
Total	33		100%

The age group (21-30) years was the most infected(39.3%) because it is the appropriate age for maturation and pregnancy and women are eligible for delivery in this age group, also the hospital was especially for women and children, while the age group (≥10) years recorded the lowest rate, the CDC also notes that infections are rising among women of childbearing age, while the virus is not always transmitted from a pregnant woman to her baby, it is possible: About 6 infants in 100 born to mothers with the virus are infected [12]. Most cases were rural areas (52%) due to the use of drug injection is common. The year of 2016 recorded the

highest infection rate (36.3%) comparing with the year of 2015 which recorded no infections.

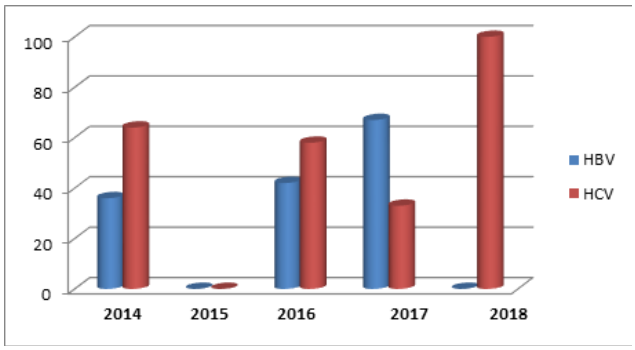


Fig (1): Distribution of HBV and HCV according to years of diagnosis

Three of the HCV cases had thalassemia as accompanying disease as hepatitis C virus (HCV) is the major cause of post-transfusion hepatitis infection (PTH). Patients with thalassemia major are at high risk of HCV(13).

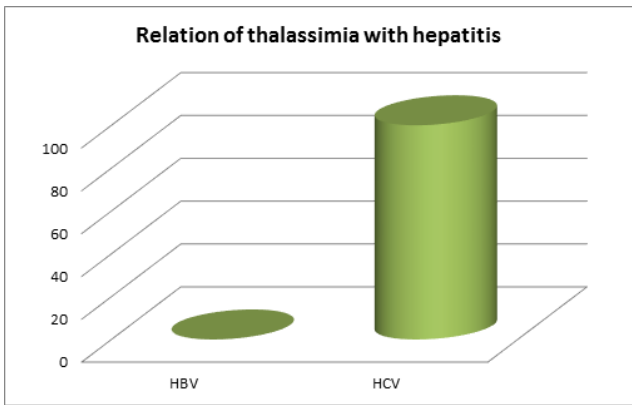


Fig (2) : Relation of thalassemia with type of hepatitis

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. World Health Organization .Viral hepatitis .EB 126/15.2009
2. Centers for Disease Control and Prevention. Morbidity and Mortality Weekly Report (MMWR). Recommendations and Reports: Public Health Service Inter-Agency. Guidelines for Screening Donors of Blood, Plasma, Organs, Tissues, and Semen for Evidence. 1991; 40: 1-17
3. Atallah, M.T. Clinical and laboratory assessment of hepatitis B surface antigen healthy carriers [thesis]. University of Baghdad: College of Medicine. 1987
4. Alter MJ. The epidemiology of acute and chronic hepatitis C. *Clin Liver Dis.* 1997; 1(3): 559-68.
5. Zuckerman AJ. "Chapter 70: Hepatitis Viruses". In Baron S; et al. Baron's Medical Microbiology (4th ed.). Univ of Texas Medical Branch. 2018; ISBN 978-0-9631172-1-2.
6. World Health Organization . Hepatitis B. Archived from the original on 2015-07-10.
7. Locarnini S."Molecular virology of hepatitis B virus". *Semin. Liver Dis*. 2004; 24 (Suppl 1): 3–10.
8. Balakrishnan L, Milavetz B."Epigenetic Regulation of Viral Biological Processes". Viruses. 2017; 9 (11): 346.
9. Ivanov AV, Valuev-Elliston VT, Tyurina DA, Ivanova ON, Kochetkov SN, Bartosch B, Isaguliants MG ."Oxidative stress, a 9.trigger of hepatitis C and B virus-induced liver carcinogenesis". *Oncotarget*. 2017; 8 (3).
10. Higgs MR, Chouteau P. and Lerat H .'"Liver let die': oxidative DNA damage and hepatotropic viruses". *J. Gen. Virol*. 2014;95 (Pt 5): 991–1004.
11. CDC. Hepatitis C Information For the Health Professional". 2010.
12. CDC, Viral Hepatitis . Statistics and Surveillance, 2018 .
13. Ataei B, Hashemipour M, Kassaian N, Hassannejad R, Nokhodian, Z & Adibi P. Prevalence of anti HCV infection in patients with Beta-thalassemia in isfahan-iran. International journal of preventive medicine, 2012;3(Suppl 1), S118-23.

The Impact of Congenital Heart Diseases on Growth Parameters in Children and Their Correlations with Leptin Levels

Fouad Shareef[1], Amal Salih Mahdi Bayee[2]

[1]*Assist. Prof. Dr. University of Kufa, College of Medicine./Iraq,* [2]*Diploma. Researcher. Al-Hilla Teaching Hospital, Neonatal Care Unit./Iraq*

Abstract

Children with congenital heart disease (CHD) prone to stunted growth by different parameters including weight, height and head circumference compared to a control groups, to accomplish such aim (110) children aged ranged (20 days – 13 years with CHD) have enrolled in this study, (83) patients with a cyanotic CHD (75.45%) and (27) cyanotic type (24.54%) were compared with (171) healthy controls of similar age and sex groups. This study was done in Al-Hilla teaching hospital during the period November 1, 2017 to October 11, 2018. The results indicate a significant (2.06) retardation in all parameters in patients with CHD related to control and there is a significant retardation in weight (z = 3.06) in children with cyanotic CHD related to a cyanotic type. Also there is a significant retardation (z = 1.01) in height gain in children with cyanotic related to a cyanotic type and finally there is a significant (z = 2.09) decrease in head circumference in children with cyanotic CHD related to a cyanotic type. Serum leptin levels were also lowered in all patients with CHD. The results concluded that children with (CHD) experience early and continues decrement of all growth parameter during their life.

Keywords: (*children, congenital heart diseases, growth parameters, leptin levels*)

Introduction

Congenital heart disease is defined as gross structural abnormality of the heart or intrathoracic great vessels that is actually or potentially of significant[(1)]. It is considered as third congenital diseases in children and a leading cause of death in infant during first year of life[(2)]. It prevalence is 5 to 8 per 1000 live births that varied in different parts of the world, in a recent study the prevalence has been reported to be ranged 4 to 50 cases per 1000 live Births[(2,14,16)].

Several genetic and environmental risk factors have been introduced for CHD, and most important factors include genetic mutation, alcohol drinking, abusing some drugs (most famous thalidomide and cocaine using during pregnancy)[(4,17)]. The cytokines in many studies were found to have a strong effect on feeding, weight and energy intake in patients with CHD[(5)] . Impaired absorption can also be an important cause of malnutrition in CHD, therefore, children with CHD and delayed growth due to increased work of a cardiopulmonary and consequently, fatigue and loss of appetite, dyspnea, tachypnea and chronic hypoxia were directed to malnutrition [(4,8,15,21).]

Recent studies shows series of serum factors such as leptin, ghrelin and tumor necrosis factor alpha (TNF-) will be changed in these patients. Consequently the rate of absorption of nutrients, growth, weight, energy consumption and storage are changing[(11)]. These children with CHD have normal growth when receiving more calories compared to healthy ones[(9,12)] and malnutrition in these patients effects on the metabolic response to injury and complications and outcomes of cardiac surgery including sepsis, renal dysfunction, necrotizing enterocolitis, hospitalization days[(13,21)].

The CHD is divided into four major groups of cyanotic with and without an increase in pulmonary artery pressure and a cyanotic heart defect with and without an increase in pulmonary artery pressure[(1,20)]. Since it has been shown that the prevalence of growth parameters retardation with CHD were increased in the

last decade in Iraqi population and since leptin hormone has been shown to be involved in long-term regulation of energy balance by suppressing appetite and stimulating weight lost[18] and such correlation has not been studied in Hilla (Babylon province), therefore, this study was undertaken to investigate:

1- The effect of CHD on anthropometries measurements of growth in children involving Weight, height and head circumference between control and cyanotic and a cyanotic CHD.

2- The variation in the effect of cyanotic and a cyanotic CHD on growth parameters.

3- Correlation of serum leptin levels with CHD.

Materials and Method

A study of (110) patients with CHD, (83) with a cyanotic CHD and (27) cyanotic CHD and (171) controls was carriedout between November 1, 2017 to October 11, 2018 in AL Hilla Teaching Hospital. These patients divided into four groups according to their age. The criteria used for including the patients in the study are:

a) Patients with cyanotic and acyanotic CHD.

b) The age of patients ranged from (20 days – 13 years).

c) Parental consent.

The patients with other congenital anomalies, with chronic disease or acquired heart diseases were excluded from the study. The control groups were selected from general paediatric and neotal care clinics, and no pathological findings had been shown in their clinical examination. The patients and control were subjected to the following:

- Complete history and physical examination.
- Anthropometric measurement including length in cm, weight in kg and head circumference in cm.
- Investigation including:

1- CXR interpreted by a radiologist

2- E.C.G. standardized at 25 mm/s

3- Echo Philips clearvue 350. USA. Used modes: M-mode, 2D and Doppler both continues and colour.

4- Blood samples were collected by venipuncture from all patients and controls. Samples were centrifuged at 3000 rpm for 20 minutes. Clear sera were separated and kept frozen at -20^{o}C until the time of assay. Leptin was measured using the DRG Elisa (DRG international, Inc., USA) by indirect enzyme linked immunosorbent assay according to the protocol provided by manufacturer. The intensity of the colour developed is proportional to the concentration of leptin in the sample, the absorbance is measured at 450 nm and concentration were determine from standard curve.

Statistical Analysis

The -Z- Statistical test was done to the significance in difference of cyanotic and a cyanotic CHD on growth parameters in comparison with control (Daniel, 2009) [19].

The level of significance taken 95%

$Z_C =$

If $Z_C > 1.96$ mean there is significant differences

Results

The hundred ten children with CHD represented by (83), 75.45% acyanotic group compared with cyanotic CHD (27) 24.54%. The patients were classified into four groups according to their age. (table 1).

Table (1): Classification of children according to age

Age	Control		A cyanotic CHD		Cyanotic CHD	
	Number	%	Number	%	Number	%
<1y	29	16.45%	43	51.80%	17	62.96%
1-2y	35	20.46%	14	16.86%	5	18.51%
2-5y	65	38.01%	14	16.86%	3	11.11%
>5y	42	24.56%	12	14.45%	2	7.4%

Table (2): The effect of CHD on head circumference

Centile	<1y			1-2y			2-5y			>5y		
	Control %	Acyanotic %	Cyanotic %	Control %	Acyanotic %	Cyanotic %	Control %	Acyanotic %	Cyanotic %	Control %	Acyanotic %	Cyanotic %
<3rd	- -	2.32	- -	- -	7.14	- -	- -	- -	- -	- -	- -	- -
3rd	3.44	30.23	17.64	- -	21.42	11.76	- -	7.14	5.88	2.38	25	50
10th	- -	16.27	17.64	2.58	7.14	5.88	1.53	28.57	- -	- -	- -	- -
25th	10.34	2.32	17.64	25.71	- -	- -	7.69	7.14	5.88	9.52	- -	50
50th	31.01	30.23	23.52	28.57	35.71	- -	56.29	21.42	5.88	40.47	58.33	- -
75th	37.97	6.97	17.64	25.71	14.28	11.76	29.23	21.42	- -	16.66	8.33	- -
95th	20.68	9.3	5.88	17.14	14.28	- -	4.61	- -	5.88	- -	8.33	- -
>95th	- -	- -	- -	- -	- -	- -	- -	- -	- -	28.57	- -	- -

Leptin levels were found to be significantly lowered in patients with cyanotic and acyanotic groups compared with controls (P<0.05), Table (3).

Table (3): Serum Leptin Levels in Cyanotic and Acyanotic CHD

	Acyanotic	cyanotic	control groups	P values
Leptin (ng/ml)	1.91.4	1.7 2.1	3.7 2.4	<0.05 P1 = 0.28 P2 = 0.02 P3 = 0.01

Data were expressed as mean standard deviation.

P_1: Acyanotic versus cyanotic groups.

P_2: Acyanotic versus control groups.

P_3: Cyanotic versus control groups.

Discussion

The study shows a statistical significant (z = 2.06) retardation in all growth parameters involving all age groups (below 1year , 1-2years, 2-5years and above 5years) but there is no significant (z = 1.2) differences between cyanotic and a cyanotic CHD in relation to any group. Table (1) shows more details, however, there is early presentation and diagnosis for children with cyanotic CHD possibly due to more aggressive clinical presentation in the infancy which attracts the parents attention to seek early medical help.

This study shows significant (z = 3.06) retardation in weight between groups of children with CHD and control, also shows significant (z = 2.04) retardation in children with cyanotic CHD, compared with a cyanotic groups, and this is an expected result due to more harmful effect of CHD (cyanotic) on the nutritional

status of children related to shortness of breath which presented as early failure to gain weight that may lead later on to growth retardation with its complications, (Figure 1) shows that weight retardation below the third centile (100%) in children more than 5 years with cyanotic CHD, also more effect of a cyanotic CHD on both groups (<1year and 1–2years, Figure 1) more than other groups (<1year, 53.48%) at the third centile and more details can be shown on (Figure 1), these findings were consistent with other reports[14], mentioned that children with CHD experience early simultaneous decrease in growth trajectory across weight, length and head circumference, this decrement suggests a role for altered growth retardation in child with CHD.

There is a statistically significant (z = 2.28) retardation in height in children with CHD in comparison with controls, but there is a non-significant (z = 1.01) variation between cyanotic and a cyanotic type. These results were also comparable with finding where (52%) below the 16th centile for both length and weight and 27% were below the 3rd centile for weight and lengths, these comparable results probably indicated that the cause of growth retardation in CHD was multifactorial which could be due to inadequate caloric intake and feeding difficulty[11], in our study there was (25.92%) of cyanotic CHD retardation in height and (42.16%) of a cyanotic type with retardation in height, (Figure 2) shows that 50% of children with cyanotic CHD above 5 years at third centile mostly in cyanotic type.

Head circumference was significantly (z = 2.56) reduced in CHD children in comparison with controls, also there was significant (z = 2.09) retardation in cyanotic type related to acyanotic, this may be explained by early affection of children with cyanotic CHD related to sever symptoms associated with sever growth retardation that lead to affection of skull development later on, (Table 2, Figure 3) shows (50%) of cyanotic CHD children aging above 5 years their head circumference on the 3rd centile comparing with acyanotic type (8.33%) reach 95th centile, also (50%) of cyanotic type below 3rd centile while there is less effect of a cyanotic CHD in early infancy, these results were also agreed with Barbara[3] who mentioned that children with CHD had stunted growth and require feeding supplementation in nearly a quarter of them (during infancy) to meet the definition of failure to thrive in first year of life[3].

Leptin is a hormone that is produced mainly by the fatty tissue and released into peripheral circulation and binds to receptors in the hypothalamus to transmit information about triglyceride content of adipocyte, in addition to macronutrient content and energy composition of newly administered food[10]. Low levels of leptin have been found to increase activity of orexigenic peptides and decrease activity of anorexigenic peptides, thereby, increasing appetite and stimulating weight gain[17]. Our study demonstrated that serum leptin levels were significantly lowered compared with controls. These results were consistent with a researcher who found that children with CHD had lowered leptin levels than healthy controls[18], while other researcher found no significant difference in plasma leptin levels between cyanotic and acyanotic patients, however, these studies did not include a healthy control groups to compare with[11,18]. This study also showed that all the anthropometric parameters in all groups were positively correlated with leptin levels. These findings were supported by others[9,11,12].

This study has some limitation since it was a single center study done in Babylon Province and in addition to hemodynamic variability associated with various types of CHD[11]. It was concluded that children with CHD are at increased risk for poor growth parameters and reduced leptin levels in these patients suggesting a role for such hormone in regulating food intake, energy balance and maintenance of body weight. The abnormal hemodynamics and the hypermetabolic state of these patients will compromise nutrition and decreases IGF-1 synthesis with subsequent slowing of linear growth weight gain[20,16].

The incidence of growth disorders in these patients can emphasized that growth retardation in patients with CHD compared with healthy children can be attributed to differences in the factors affecting the growth of CHD children including gender, age, cardiac abnormalities simultaneous multiple valvular lesions and ultimately congestive heart failure that leads to multiple growth problems[3,17].

Conclusions

- There was significant decrease in all growth parameters (z = 2.06) in children with CHD related to control.

- There was a significant retardation in weight (z = 2.09) and head circumference (z = 2.09) between cyanotic and a cyanotic CHD

- There was a non-significant retardation (z = 1.01) in height between cyanotic CHD and a cyanotic CHD.

- Serum leptin level were lowered in both cyanotic and a cyanotic patients with CHD.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1- Karen J.M.; Robert, M.K., Hal, B.J. Cardiouscular system, Chap. 19. ln: essentials of pediatrics (Nelson) 6th ed. (2006). Pp. (525-557).

2- Malnutrition and growth failure in cyanotic and acyanotic congenital heart disease with and without pulmonary hypertension. British prevalence of CHD word wide (November, 2018). Online.

3- Barbara, M: nutrition and growth in CHD challenge in children. International Journal of Pediatrics (2017). 29(11): 1182-1190.

4- Noor, M; Noori, J.; Maryam, N.: Evaluation of growth status with CHD: a case control study. International journal of paediatrics (2017). 5(12): 6503-6514.

5- AliMehrizi, M.D and Allan, M.D. "Growth disturbance in congenital heart disease." International Journal of pediatrics (2017) 61(3): 5603-5620.

6- Noor NM, Sadeghi, shahramian, I; keshavarz, k. Urine B_2 – Microgloblin in the patients with congenital heart disease. Int. Cardiovasc. Res J. (2013). 7:62-6.

7- Jenkins, KJ: Non inherited risk factors and congenital cardiovascular defects current knowledge a scientific statement from the American Heart Association Council on cardiovascular disease in the young: endorsed by the American Academy of pediatrics. Circulation. (2007). 115(23): 2995-3014

8- Noor, NM; Teimouri A; Boryri T; Risbaf Fakour S; Shahramiau, F. Incidence of Congenital Heart disease. Anomalies in Newborns with oral clefts in Zahedan. Iran International Journal of Pediatric. (2016). 4(9): 3363-71.

9- Siwki Es, Erenberg F., Zahkak G, Goldmuntz E, In: Alen HD, Clark EB , Gutgesset HP, editors. Moss and Adam's Heart disease in infants, children and Adolescents. Philadelphia USA: Lippinocott Williams and Wilkins. (2008).

10- Shahramian, I; Noori, NM; Hashimi, M; Sharafi, E; Baghbanian, A.: A Study of Serum Levels of Leptin, Ghrelin and Tumor necrosis factor alpha in child patients with cyanotic and a cyanotic congenital heart disease. J. Pak. Med. Assoc. (2013). 63(11): 1332-37.

11- Varan, B; Tokel, K; Yilmaz, G. Malnutrition and growth failure in cyanotic and acyanotic congenital heart disease with and without pulmonary hypertension. Archives of diseases in childhood. (1999). 8(1): 49-52.

12- Oyarzun G, Guillerm. Nutritional Rehabilitation for children with congenital heart disease with left to right shunt , 2018, 23 (15): 55 62.

13- Chris Raab, growth and development with end organ failure. Solid organ transplantation in springer infants and children. (2017). 6(18): 113-118

14- Carrie D., Ashley N. Growth in Children with congenital heart disease pediatrics January 2013 Volume 131 ISSUE 1 Article.

15- Onism D., Garaza C. WHO growth standards for infants and young children. Arch Pediatr., 2009, Jan. (207-225)

16- Ashraf, TS; Ahmed, M; Abdelgall, M and Said, MA. Growth parameters and endocrine function in relation to echocardiographic parameters in children with VSD without heart failure. J. of Tropical Paediatrics. (2001). 47(3): 146-152.

17- Olgu H; Dursun A; Nurgun, K. Plasma leptin levels in children with cyanotic and a cyanotic congenital heart disease and correlation with growth parameters. International Journal of cardiology (2003). 92 (1): 93-97.

18- Rania, SZ.; Wael, AB.; Safaa, T. Changes of serum leptin levels and ghrelin levels in children with CHD and correlation with growth parameters changes. Research Journal of Medicine and Medical Science. (2018). 13(1): 27-34.

19- Danial, WW.; (2009). Biostatistics: A foundation for analysis in the health and sciences. John Willy and Sons. Inc. 9th ed.

20- Varan, B.; Tokel, K.; Yilmaz, G. Malnutrition and growth failure in cyanotic and a cyanotic heart disease with and without pulmonary hypertension. Archives of disease in childhood. (1999). (1): 49-52.

21- Daymont, C; Neal, A; Prosnitz, A; and Cohen, MS. Growth in children with congenital heart disease. Paediatrics. (2013). 131(1):236-242.

Hormonal and Mineral Imbalance Effect on Bone Resorption in Predialysis Iraqi Patients with Chronic Kidney Disease

Inaam Ahmed Ameen[1], Sarah Hashim Mhaibes[2], Shahad Wisam Ahmed[3], Kaled Nather Taha[4] And Dhikra Abdul Hameed Dawood[5]

[1]Assistant Professor, [2]Assistant Lecturer, [3]Pharmacist / Department of Clinical Laboratory Sciences, College of Pharmacy, University of Baghdad, Baghdad, Iraq, [4]Clinical Immunologist, [5]Clinical Biochemist, Ministry of Health / Medical City / National Center for Educational Laboratories, Baghdad, Iraq

Abstract

Introduction: Chronic kidney disease mineral bone disorder is a metabolic bone disease present in almost all uremic patients. The aim of this research to indicate the stage of chronic kidney disease (CKD) that affect the bone metabolism that leading to the mineral and hormonal imbalance by studying the relationship among osteocalcin , glomerular filtration rate (GFR), parathyroid hormone, calcium and phosphorus levels in the blood.

Method: The study included 52 patients with predialysis chronic kidney disease stage 3-5 and 40 apparently healthy relatives accompanying the patients. Glomerular filtration rate (GFR) was calculated for each patient. Renal function tests, including serum levels of urea, creatinine, a biochemical marker of bone metabolism: osteocalcin (OSN), calcium, phosphorus, and parathyroid hormone (PTH), were measured for each participant.

Results: Serum urea and creatinine levels were significantly higher in CKD patients Than that of apparently healthy control. There is significantly higher serum parathyroid hormone, serum phosphorus, serum osteocalcin ($P<0.01$, $P<0.01$, $P<0.01$ respectively) in CKD patients than that of the healthy control group. While low serum calcium level in CKD patients as compared to the corresponding group ($P<0.01$).

Conclusion: Hyperphosphatemia and hypocalcemia in the end stage of predialysis CKD patients lead to increase parathyroid hormone secretion, which causes high bone turnover characterized by significantly high serum osteocalcin in these patients. Parathyroid hormone and osteocalcin were used as a biomarker for the development of bone and mineral disorders in predialysis CKD patients.

Keywords: *CKD, parathyroid hormone, osteocalcin, calcium, phosphorus*

Introduction

Chronic kidney disease (CKD) has become a public health problem. The definition of CKD was introduced by National Kidney Foundation in 2002 and later adopted by the international group Kidney Disease Improving Global Outcomes in 2004, a decrease in kidney function with a glomerular filtration rate (GFR) < 60 mL/min per 1.73 m^2 and/or kidney damage for 3 months or more [1] . Chronic kidney disease, mineral bone disorder (CKD-MBD) is a metabolic bone disease present in almost all uremic patients. With uremia, bone is relatively resistant to parathyroid hormone (PTH) action, such that the average level of PTH is required to maintain bone turnover [2]. Relative hypoparathyroidism is associated with low-turnover or a dynamic bone disease [3]; severe secondary hyperparathyroidism leads to high turnover bone disease [4]. Secondary hyperparathyroidism occurs in CKD produces an imbalance between osteoclast activity and osteoblast synthetic activity ,leading to enhanced bone breakdown at the end stage of renal disease [5].

Corresponding Author:
Sarah Hashim Mhaibes
E-mail: phsarahhashim@gmail.com

Osteocalcin(OSN) is a non-collagenous, vitamin K-dependent protein with 46–50 amino acid, produced by osteoblasts, it is a marker of bone formation [6]. High blood OSN levels are also in older adult humans, high levels in the blood are a predictor of lower bone density and a sign of fracture risk, including hip fractures and this because OSN levels can increase in the blood as a result of the breakdown of bone tissue [7]. The circulated OSN is removed by the kidney and liver[8], which increase in the blood when the renal function is declining. Patients with CKD show a progressive increase in serum OSN levels that closely corresponded with intact PTH and alkaline phosphatase levels. More fundamentally, such increases in serum OSN levels reflect the severity of the bone lesions [9].

Small increases in serum OSN were found in some patients at a severe stage, while a significant increase in blood OSN is shown in patients with end stage predialysis. In such patients, this elevation either due to decreased renal clearance of OSN or also reflected increased bone metabolism [10,11]. There is a negative correlation between glomerular filtration rate (GFR) and plasma osteocalcin levels in predialysis patients [12].

The aim of this research to indicate the stage of chronic kidney disease that affects the bone metabolism that leading to the mineral and hormonal imbalance by studying the relationship among OSN, GFR, PTH, calcium and phosphorus levels in the blood.

Method

The case-control study was conducted from March to Jun 2019, at the National Center of Teaching Laboratories of Medical City Institute, Baghdad, Iraq. Data of predialysis CKD Iraqi patient's attendant to Ghazi Alhariry hospital in Medical City in March 2019 for renal evaluation function were included in the study. The study was approved by the Ethics Committee of the University of Baghdad, Faculty of Pharmacy(UBCP-RECA-M62019A). 52 patients with predialysis chronic kidney disease stage 3-5 were enrolled, and 40 apparently healthy relatives accompanying the patients were selected. The purpose of the study and nature of all procedures were explained to participants, and informed approval was obtained before the commencement of the study. Patients were excluded if they had an acute infection, cancer, acute myocardial infarction, pulmonary edema, and patients on medication (steroid, bisphosphonates, calcium or vitamin D). The diagnosis of predialysis CKD patients was made by nephrologist based on the estimation of GFR together with renal function tests [table1]. GFR was calculated by the Modification of Diet in Renal Disease(MDRD) equation: 186 x (Creatinine/88.4)-1.154 x (Age)-0.203 x (0.742 if female) x (1.210 if black). Moderate reduction of GFR (30–59 mL/min/1.73 m^2) in stage 3, severe reduction of GFR (15–29 mL/min/1.73 m^2) in stage 4 preparation for renal replacement therapy and established kidney failure or end-stage renal disease (ESRD) (GFR <15 mL/min/1.73 m^2) in stage 5 requiring permanent renal replacement therapy (RRT).

The serum OSN was determined by Chemiluminescent enzyme immunoassay using Immulite 1000 autoanalyzer [13] (LKON1Siemens, USA). Serum urea nitrogen was measured by using urease/glutamate dehydrogenase coupled enzymatic technique (Dimension clinical chemistry System ,DF21 Siemens, USA). Serum creatinine was measured by using modified kinetic Jaffe technique (Dimension clinical chemistry System, DF33B Siemens, USA). Serum intact PTH was measured by using two-site chemiluminescent enzyme-labeled immunometric assay [14] (DPC Immulite 2000, Siemens, USA). Serum calcium and phosphorus levels were quantified by using Ca(DF23A), PO_4 (DF61A) Dimension Rxl Siemens autoanalyzer as per International Federation of Clinical Chemistry (IFCC) guidelines, modifications of calcium O-cresolphthalein complex one reaction (OCPC) and classical phosphomolybdate method, respectively [15,16].

Statistical Analysis

It was performed by using the SPSS Statistics version 20.0 The results were expressed as mean and standard deviation. Results were analyzed utilizing One-way ANOVA was used to determine the significance degree between parameters. The p-value ≤ 0.05 was considered significant. ROC curve was used to identify the validity of markers as an indicator of the disease.

Results

Serum levels of both urea and creatinine levels were significantly higher in CKD patients than apparently healthy control. These results were used together with the estimated GFR (< 60 mL/min per 1.73 m^2) to determine the stage of CKD in patients group [table 1] .

Table 1: Descriptive statistics between stages of CKD patients and healthy control.

Parameters	Stages	N	Mean	Std. Deviation	ANOVA test (P-value)
Age / Year	A.H. Control	40	44.61	11.505	P=0.00*
	3	12	63.60	2.510	
	4	20	58.95	5.781	
	5	20	49.85	10.713	
	Total	92			
Serum Urea	A.H. Control	40	29.67	3.029	P=0.00*
	3	12	52.20	9.445	
	4	20	67.55	11.651	
	5	20	191.85	71.582	
	Total	92			
Serum Creatinine	A.H. Control	40	0.805	0.123	P=0.00*
	3	12	1.740	0.288	
	4	20	2.760	0.454	
	5	20	9.230	4.123	
	Total	92			
GFR	A.H. Control	40	97.78	22.709	P=0.00*
	3	12	43.40	8.764	
	4	20	21.80	4.312	
	5	20	6.75	2.971	
	Total	92			
OSN	A.H. Control	40	6.12	7.012	P=0.00*
	3	12	11.90	8.389	
	4	20	26.21	16.848	
	5	20	41.04	22.372	
	Total	92			
Serum Calcium	A.H. Control	40	9.42	0.405	P=0.00*
	3	12	8.60	0.424	
	4	20	7.98	0.714	
	5	20	6.89	0.824	
	Total	92			
Serum phosphorous	A.H. Control	40	3.11	9.285	P=0.00*
	3	12	3.50	1.027	
	4	20	4.38	1.071	
	5	20	5.92	1.279	
	Total	92			
PTH	A.H. Control	40	22.33	6.791	P=0.00*
	3	12	40.60	14.311	
	4	20	60.35	19.906	
	5	20	94.70	23.939	
	Total	92			

*A.H : apparently healthy, CKD: chronic kidney disease, GFR: glomerular filtration rate, OSN: osteocalcin, PTH: parathyroid hormone Statistically significant at p <0. 05 , statistically highly significant at p-value ≤ 0.01 and non-significant at p>0.05

Curves of ROC studies confirmed that the blood urea and serum creatinine were a highly sensitive and specific diagnostic marker of CKD [figure1]. The sensitivity, specificity and cut-off point of hormonal and minerals for predialysis patients with CKD were estimated by ROC [table 2]

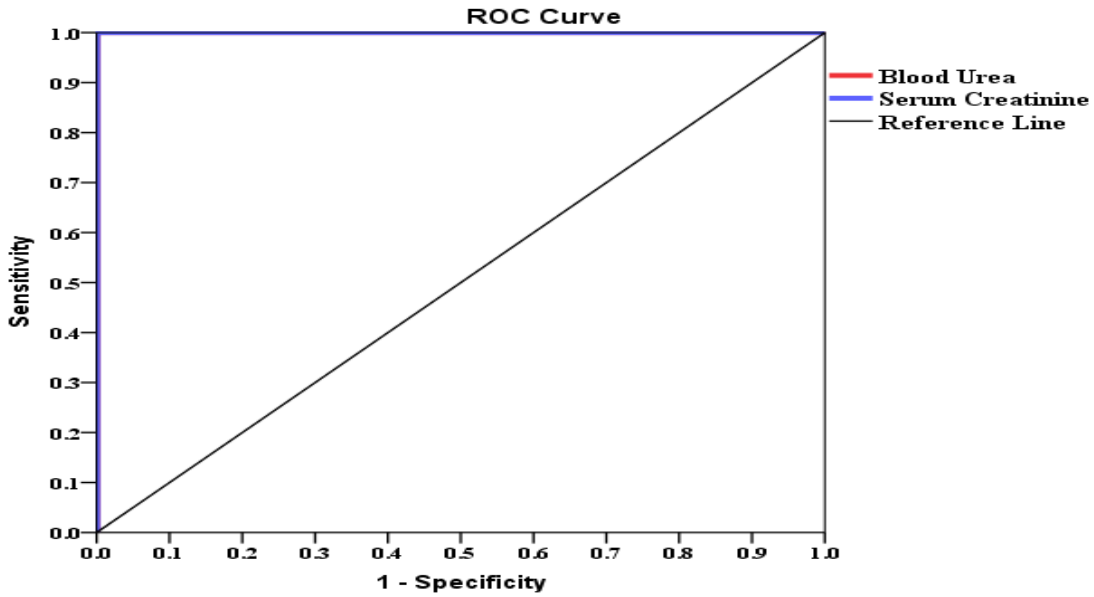


Figure 1: ROC for serum urea and creatinine levels

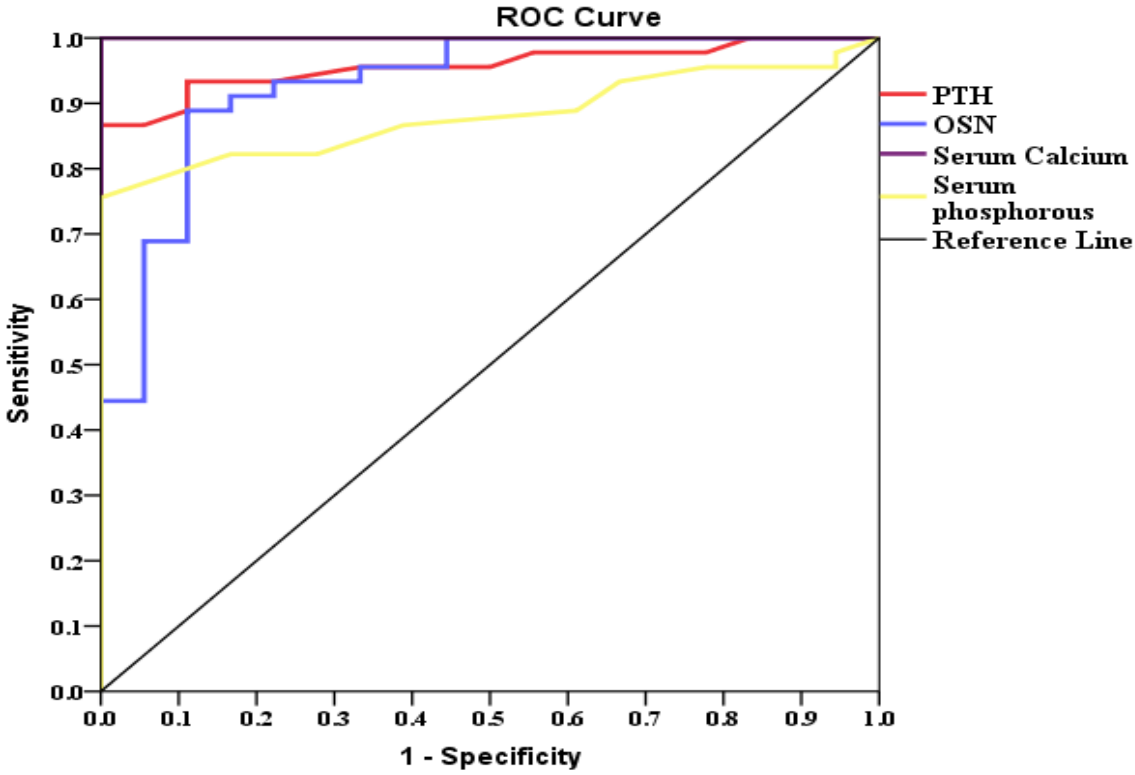


Figure 2: ROC curves for PTH, OSN, serum Calcium and serum phosphorus

Table 2: The cut-off point with sensitivity 100, specificity 100 and Area under the curve (AUC) 1.000 of laboratory results

Validity tests	Serum urea	Serum Creatinine	Serum Calcium	Serum phosphorous	OSN	PTH
Sensitivity	100%	100%	80%	86.7%	91.1%	95.6%
Specificity	100%	100%	100%	61.1%	77.8%	50%
Area Under the curve (AUC)	1	1	0.972	0.881	0.928	0.957
Cutoff value	> 38	> 1.2	< 8.5	> 3.1	> 6.6	> 21.5
P-value	0.00 HS	0.00 HS	0.00 HS	0.00 HS	0.00 HS	0.00 HS

HS=Highly significant difference (P<0.01)

Discussion

In the present study, the end stage of CKD patients has significantly higher serum concentration of the urea and creatinine as compared to stages 3-4 of CKD patients which is in agreement with other study[17]. This is due to the progressive reduction of GFR at the end stage of CKD patients. As the GFR decreases blood levels of both urea and creatinine are increased [18]. Significantly higher serum PTH levels at the end stage of CKD group among all studied patients groups, which is agree with the other findings which reported that the serum PTH was significantly higher in more advanced renal failure (stage 5 CKD), which confirms the relationship between severity of hyperparathyroidism and the degree of renal impairment [19,20]. Secondary hyperparathyroidism result from a decreased renal function, which is a common complication of CKD that leads to an overproduction of PTH caused by several changes that occur in bone and mineral metabolism because of decreased kidney function [21]. In this study, the CKD patients have hyperphosphatemia and hypocalcemia that leads to significant hyperparathyroidism when compared to healthy control patients with normal renal function.

At the end stage of CKD, when the remaining nephrons can no longer sufficiently excrete the phosphorus load, hyperphosphatemia is detected. The calcium and phosphorus form an insoluble complex in serum. This process may lead to extraskeletal calcification and potentially calciphylaxis or cardiac disease [22]. Retention of phosphorus also indirectly causes excessive production and secretion of PTH through lowering of ionized Ca^{2+} and by suppression of calcitriol production [19]. Vikrant S et al. was found increase serum PTH level in the end stage of CKD inversely correlated with GFR and serum calcium and positive correlation with serum phosphorus which is in agreement with this study [23]. Hyperphosphatemia is recognized as the primary initiator of the various cascades of the promoters of renal bone disease [24].

The present study shows increment serum osteocalcin levels associated with progressing stage of CKD up to higher concentration in the end stage CKD . Rix et al. who reported that patients with predialysis CKD had elevated serum levels of OSN with the more severe stage of CKD corresponding to the level of secondary hyperparathyroidism [25] as in agreement with this study.

In patients with impaired renal function plasma OSN levels are markedly elevated due to increased bone turnover and decreased renal elimination [26]. The effects of increased parathyroid hormone (PTH) resultant

resistance to adaptive stimulation of bone formation by parathyroid hormone, permits the effects of kidney injury to inhibit bone formation despite the development of secondary hyperparathyroidism [27].

Phosphate retention, hypocalcemia, and bony resistance to the action of PTH all these factors may contribute to overactivity of parathyroid gland to increase synthesis and secretion of PTH in end stage CKD. High levels of OSN in blood at the end stage of CKD occurs due to elevated PTH which stimulate bone demineralization that characterized by accelerated rates of bone absorption and resorption.

Conclusion

Hyperphosphatemia and hypocalcemia in the end stage of predialysis CKD patients lead to increase parathyroid hormone secretion, which causes high bone turnover characterized by significantly high serum osteocalcin in these patients. PTH and OSN were used as a biomarker for the development of bone and mineral disorders in predialysis CKD patients. It is recommended that attending physicians monitor and control biochemical parameters early in the development of CKD before the need for dialysis to protect the CKD patients from any complications that will result in response to PTH.

Acknowledgement: The authors are grateful to National Center of Teaching laboratories of Medical City Institute, Baghdad for providing laboratory facilities for this research.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. 1. Lamb EJ, Levey AS, Stevens PE. The Kidney Disease Improving Global Outcomes (KDIGO) guideline update for chronic kidney disease: evolution not revolution. Clin Chem 2013; 59: 462-465 .
2. Tolouian R, Gupta A. The need for a reliable bone biomarker to better assess chronic kidney disease mineral and bone disorder. Journal of Parathyroid Disease. 2018;6(2):36-38
3. Sherrard DJ, Hercz G, Pei Y, Segre G. The aplastic form of renal osteodystrophy. Nephrol Dial Transplant. 1996;11:29-31.
4. Hruska K. New concepts in renal osteodystrophy. Nephrol Dial Transplant. 1998; 13:2755-ed60.
5. Cozzolino M, Brancaccio D, Gallieni M, Galassi A, Slatopolsky E, Dusso A: Pathogenesis of parathyroid hyperplasia in renal failure. J Nephrol. 2005; 18:5–8.
6. Yamanouchi D, Takei Y, Komori K. Balanced mineralization in the arterial system: Possible role of osteoclastogenesis/osteoblastogenesis in abdominal aortic aneurysm and stenotic disease. Circ J. 2012; 76:2732-7.
7. Suzuki Y, MaruyamaNagao A, Sakuraba K, Kawai S. Level of serum undercarboxylated osteocalcin correlates with bone quality assessed by calcaneal quantitative ultrasound sonometry in young Japanese females. Experimental and therapeutic medicine. 2017;13(5):1937-43.
8. Taylor AK, Linkhart SG, Mohan S, Baylink DJ. Development of a new radioimmunoassay for human osteocalcin: evidence for a midmolecule epitope. Metabolism 1988; 37:872–7.
9. Coen G, Mazzaferro S, Bonucci E, Taggi F, Ballanti P, Bianchi AR, et al. Bone GLA protein in predialysis chronic renal failure. Effects of 1, 25 (OH) 2D3 administration in a long-term follow-up. Kidney international. 1985;28(5):783-90.
10. Razzaque M. S. Osteocalcin: a pivotal mediator or an innocent bystander in energy metabolism?. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association, 2011:26(1), 42–45.
11. Delmas Pd, Wilson Dm, Mann Kg, Riggs Bl. Effect of renal function on plasma levels of bone Gla-protein. The Journal of Clinical Endocrinology & Metabolism. 1983;57(5):1028-30.
12. Yamada S, Inaba M, Kurajoh M, Shidara K, Imanishi Y, Ishimura E, et al. Utility of serum tartrate-resistant acid phosphatase (TRACP5b) as a bone resorption marker in patients with chronic kidney disease: independence from renal dysfunction. Clinical endocrinology. 2008;69(2):189-96.
13. FassbenderWJ, Steinhauer B, Stracke H, Schumm-

Draeger P-M, Usadel K-H. Validation of a new automated immunoassay for measurement of osteocalcin. Clin Lab. 2002;48:31-38.

14. Lepage R, Whittom S, Bertrand S, Bahsali G, D'Amour P. Superiority of dynamic over static reference intervals for intact, midmolecule, and C-terminal parathyrin in evaluating calcemic disorders. Clin Chem 1992;38:2129-35.
15. Stern J, Lewis WHP. The colorimetric estimation of calcium in serum with O-cresolphtalein complexone . Clin Chim Acta. 1957; 2:576-80.
16. Daly JA, Ertingshausen G. Direct method for determining inorganic of phosphate in serum with the centrifichem. Clin Chem. 1972; 18:263-65.
17. Nisha R, Srinivasa Kannan SR, Thanga Mariappan K, Jagatha P. Biochemical evaluation of creatinine and urea in patients with renal failure undergoing hemodialysis. J Clin Pathol. 2017;1(2):1-5.
18. Kamal A. Estimation of blood urea (BUN) and serum creatinine level in patients of renal disorder. IJFALS. 2014;4(4):199–202.
19. Malawadi BN, Suma MN, Prashant V, Akila P, Anjalidevi BS, Manjunath S. Secondary hyperparathyroidism in all the stages of chronic kidney disease in southern indian population. IJPPS 2014; 4.;6.
20. Palmer SC, Hayen A, Macaskill P, Pellegrini F, Craig JC, Elder GJ, et al. Serum levels of phosphorus, parathyroid hormone, and calcium and risks of death and cardiovascular disease in individuals with chronic kidney disease: a systematic review and meta-analysis. Jama. 2011;305(11):1119-27.
21. Nigwekar SU, Tamez H, Thadhani RI. Vitamin D and chronic kidney disease–mineral bone disease (CKD–MBD). BoneKEy reports. 2014;3.
22. Block GA, Port FK: Calcium phosphate metabolism and cardiovascular disease in patients with chronic kidney disease. Sem Dialysis.2003;16:140–147.
23. Vikrant S, Parashar A. Prevalence and severity of disordered mineral metabolism in patients with chronic kidney disease: A study from a tertiary care hospital in India. Indian journal of endocrinology and metabolism. 2016 ;20(4):460.
24. Llach F. Hyperphosphatasemia in end stage renal disease patients: pathophysiological consequences. Kidney Int. 1999; 56: S31–S37
25. Rix M, Andreassen H, Eskildsen P, Langdahl B, Olgaard K. Bone mineral density and biochemical markers of bone turnover in patients with predialysis chronic renal failure. Kidney international. 1999. 1;56(3):1084-93.
26. Csiky B, Sági B, Peti A, Lakatos O, Prémusz V, Sulyok E. The impact of Osteocalcin, Osteoprotegerin and Osteopontin on arterial stiffness in chronic renal failure patients on hemodialysis. Kidney and Blood Pressure Research. 2017;42(6):1312-21.
27. Sabbagh Y, Graciolli FG, O'Brien S, Tang W, dos Reis LM, Ryan S, et al. Repression of osteocyte Wnt/β-catenin signaling is an early event in the progression of renal osteodystrophy. Journal of Bone and Mineral Research. 2012;27(8):1757-72.

Robust Controller Electromyogram Prosthetic Hand with Artificial Neural Network Control and Position

Saygin Ahmed[1], Aydin S. Ahmed[2], Bülent Yilmaz[3], Nuran Dogru[1]

[1]College of Engineering- University of Gaziantep-Turkey, [2]Technical College of Kirkuk- Northern Technical University-Iraq, [3]College of Engineering -University of Abdullah gül, Turkey

Abstract

In this study, we proposed and designed a new control method for an electromyographically (EMG) controlled prosthetic hand. The objective is to increase the control efficiency of the human–machine interface and afford greater control of the prosthetic hand. The process works as follows: EMG biomedical signals acquired from Myoware sensors positioned on the relevant muscles are sent to the robot that consist of hand, Arduino and MATLAB program, which computes and controls the hand position in free space along with hand grasping operations. The Myoware device acquires muscle signals and sends them to the Arduino. The Arduino analyzes the received signals, based on which it controls the motor movement. In this design, the muscle signals are read and saved in a MATLAB system file. After program processing on the industrial hand which is applied by MATLAB simulation, the corresponding movement is transferred to the hand, enabling movements, such as, hand opening and closing according to the signal stored in the MATLAB system. In this study, hand and fingerprints were designed using a three-dimensional printer by separate recording finger and thumb signals. The muscle signals were then analyzed in order to obtain peak signal points and convert them into data. These results indicate the effectiveness of the proposed method and demonstrate the superiority of the method for amputees because of the improved controllability and perceptibility afforded by the design.

Keywords: *Arduino controller., Electromyography, Hand robot, Prosthetic hand.*

Introduction

Nowadays, the development of science and technology has led to prosthetic devices with promising functional capabilities and esthetic appearance in research domain in favor of commercialization[1].

The design of prosthetic hand is multidisciplinary, compelling knowledge of physiology, anatomy, electrical and electronics, mechanical design, software, and so on, depending on the nature of control Robotic prosthetic hands have attracted considerable attention in terms of their practical use by amputees. Artificial neural network is used to classify the signal features and subsequently recognize the performed movement [2]. Along this research line, Sumit et al. conducted real-time identification of active hand-movement EMG signals based on wrist-hand mobility for simultaneous control of prosthetic robotic hands [3]. In another system, a fully wireless, mobile platform used for acquisition and communication of sEMG signals is embedded in a mobile control system, and Ottobock 13E200 EMG electrodes are used to acquire the EMG signals. The electrodes are attached to the patient's remaining forearm stump. In addition, a laptop is used to provide the required computational power for the control of the prosthetic robotic hand [4]. In the light of reducing costs, some studies have utilized an open-source design for the implementation of affordable, modular, compliant, under-actuated prosthetic fingers that can aid amputees who suffer from partial amputations (e.g., amputations of one or several fingers of the human hand, with the exception of the thumb) to regain lost dexterity [5]. In general, the control design of a robotic arm employs fuzzy algorithms to interpret EMG signals from the flexor carpi radialis, extensor carpi radialis, and biceps brachii

Corresponding Author:
Aydin S. Ahmed
E-mail: aydinfedakar@ntu.edu.iq

muscles. In one type of control approach, the control and acquisition system consists of a microprocessor, analog filtering, digital filtering and frequency analysis, and a fuzzy control system, and electromyographic grasp recognition together with an 8-bit microcontroller is used to control a veneered robotic hand to emulate six grasp types that are used for over 70 % of daily activities [6,7]. A new configuration of sEMG electrodes has been reported to reduce interference resulting from electrode shift depending on muscles movement [8]. The authors suggested that optimizing electrode configuration can improve the EMG pattern discrimination, wherein the proposed electrode configuration has a reference value.

Myoelectric prosthetic hands are primarily intended for adults but are also made by many companies for commercial purposes as prosthetic hand for children[9,10] .

It is also noteworthy that almost all robotic hands designed in university research projects consist of numerous actuators and sensors, which makes them unsuitable for manufacturing along with being too expensive for the typical user. In general, the medical industry can greatly benefit from providing low-cost portable systems that allow visualization of patient data easily and remotely while also providing quick access to accurate data in real time, thereby enhancing the efficiency of doctors and specialists along with providing the patient with greater ability and care.

Today, robotics considered as one of the best technologies that deal with design, working and applications of robots, computer systems their control and and information processing. These technologies help physiotherapists and robotics engineers to model and design robotic hands. We aim something more than material and physical. As a result, we want to create opportunities for people with no hands and ability to last their daily lives.

Aim and Objectives

In this study, hand and finger prints were designed using a three-dimensional printer by separate recording finger and thumb signals. The muscle signals were then analyzed in order to obtain peak signal points and convert them into data. These data are classified according to muscular positions and used for hand control

Materials and Method

Mechanical Hand Design

The prototype hand used in the study was a 3D printed version of the Flexy Hand[11]. Therefore, the corresponding STL file was exported into the Makerbot platform and directly printed without any scaling or modification. Along with the separate parts of the hand, the printing of the entire hand took about 11 hours to be completed. The completed hand was strung with a fishing line and a stretched disposable pipette. The disposable pipette was used to clear any excess material from the 3D printing that would hinder the fishing line's path through the interior of the hand and fingers, and to aid in threading the line through the palm of the hand. Each finger was strung with about two feet of fishing line to ensure that there would be sufficient material to reach down the length of the arm and attach to the servos.

Motor Control

A servo motor with three wires, power wire, ground wire, and pulse-width modulation (PWM) wire, was used to drive the hand. The PWM wire was connected to one of the six PWM ports of an Arduino UNO board. The power and ground wires of each servo were connected to the horizontal positive and negative rows on the breadboard that was connected to a 6-V battery pack. The battery pack housed four 1.5-V D-size batteries. The PC module was plugged into the USB cable.

Hand Control

In this section, we used an Arduino UNO unit in this study to analyze the EMG signals acquired from the muscles. The signal is handled by the motor, and the UNO board is also used to send PWM information to the motor for hand control. By Using Myo arm band to provide the signal for MATLAB for recording it and simulating the signal after passing from equalization then the command which has been created by MATLAB will be pass to the microcontroller which has the all control to the hand by using servos.

Another benefit of having a simulation of the hand model is that it allows representing the results as functions of parameters (such as, the weight or type of material) to work on further improvements. Lastly, the grasp quality and optimization of the finger positions for different grasps are other crucial aspects that can be tested with a good model. The Simulink program is designed for multidomain simulation and model-based design. As mentioned previously, Simulink has the ability to simulate and generate automatic code, and allows conducting various tests along with verification

of the embedded systems. Further, Simulink enables users to incorporate their MATLAB algorithms into models and export the simulation results to MATLAB for additional analysis.. The Simulink program consists of the following commands: constant, Slider Gain, (Sum, Add, Subtract, and Sum of Elements), Sine Wave, and Arduino IO servo Write. The constant is used for generating a real or complex constant value. The Slider Gain is used for varying the scalar gain during the simulation by using the slider; this block has one input and one output. The sine wave is used for generating a sinusoidal waveform, thus indicating that the output of this block is sinusoidal. The sine wave and Slider Gain signals are directed to the mixer, whose output is the summation of the sine wave and Slider Gain signals. The output of the mixer forms the input of the servo Write.

EMG Signals

In this study, we used an EMG shield to obtain the signals from the arm muscles, and because the signals were not very clear, we used another high-sensitivity device to obtain clear signals.

$$y = \frac{1}{N}\sum_{i=1}^{N} |Xi| \quad \text{.....(1)}$$

Where N is the length of the signal and X_k represents the EMG signal in a segment. A simple way to measure the level of muscle activity is absolute value and this feature is common for use in myoelectric control. This feature is used for all classification in this project.

Root mean square:

$$y = \frac{1}{N}\sqrt{\sum_{i=1}^{N} X^2}$$

……..(2)

Slope sign change:

……(3)

Waveform length:

$$y = \sum_{i-1}^{N-1} (|X_{i+1} - Xi|) \quad \text{……..…….(4)}$$

Zero crossings:

$$y = \sum_{i_1}^{N-1} f(X)$$

…….(5)

Willson Amplitude:

$$y = \sum_{i_1}^{N-1} f(|X_{i+1} - X_i|)$$

…….(6)

Variance:

$$y = \sum_{i_1}^{N-1} f(|X_{i+1} - X_i|)$$

$$y = \frac{1}{N-1}\sum_{i=1}^{N} (X^2) \quad \text{……..}$$

(7)

Cost Analysis

Table 1 lists the cost breakdown of the entire system, including the price of 3D printing. The prices of electronic components (resistors, capacitors, wires, solder), and mechanical component (screws, nuts, bolts, crimps, silicone) are not listed since they can be acquired easily from campus laboratories and machine shop. The cost of the project stayed well within the estimated limit. Compared to the existing advanced robotic hands that are available on the market, which cost around 90,000 $, a 350 $ robotic hand solution seems more viable to the general population of users.

Results and Discusion

In this section, we compared the performances of our device and other existing devices in the market. Here, we remark that while our hand design is based on the ideas underlying the normally manufactured industrial robotic hand, we have also added a servo motor and EMG-based grip control in order to improve the device performance as well as ensure weight reduction relative to the weights of previously manufactured hands. The full weight of our device is 500 g, and the cost is as low as $250. These benefits are possible due to the use of EMG and grip control. The table 2 compares our device

with certain other devices in the market. Comparison parameters include type, weight, and grip pattern along with other key parameters. From the table, it is obvious that our device is more feasible for practical application than other existing devices. In terms of device weight, our hand lies in the weight range of the myoelectrically controlled powered hand prosthetic and the BeBionic (RSL Stepper), whose weights are considered suitable for prosthetic hands. Overall, our findings indicate the proposed robotic hand delivers a satisfactory performance, particularly in terms of improved controllability and perceptibility over other devices. An added benefit is the fact our device is less expensive than other devices.

Due to an experimental limitation and difficulties problems or complications with reliably dependably performing each every gesture, different users had the latency check was solely done on one user. Every gesture was performed five times from an amount of rest and control till the motion completion time may well be determined resolute whereas the myogram activity and sophistication labels tags were unendingly recorded. Table 3 shows the results of testing the hand on 7 persons who were missing an arm. The data set is for two movements; namely, the closing and opening of the hand. Some of the results have different rates of errors due to errors beyond the control of the designed system. One of common reason are the differences in the human muscles.In general, with regard to the design of robotic prosthesis, the primary challenge involves developing a flexible experimental setup for closed loop control of a prosthetic device with integrated augmented reality that allows changing the extent and type of provided visual and vibrotactile feedback. Several studies have focused on the control approach as well. In one study, thirteen volunteers participated in the experiments by controlling the Ottobock Sensor Hand Speed prosthesis [12]. The results indicated that the recorded vibrotactile patterns were able to replace visual feedback . In another study, in a multi-sensory, five-fingered Bio-Mechatronic hand with an sEMG interface, each finger was integrated with torque and position sensors that offered the hand more grasping patterns and complex control methods[13]. Arduino code could be uploaded to an online repository and made freely available for download. This code would incorporate controller programming, and a Graphical UI (GUI) that would enable the client to tune the control calculation to their inclination. The client would then have the option to purchase modest diversion gadgets, transfer the product themselves to the controller, and introduce the controller into the Talonhand themselves[14].

Table 1. Cost breakdown of all the materials used in the 3D printed robotic hand

.Item	Part No.	Price $	Quantity	Total $
Printing	Vero Blue	0.10	450 g	45.00
Printing	PLA White	0.10	40 g	4.00
Microcontroller	Arduino UNO	24.00	1	24.00
Servo	HK1529	15.00	5	75.00
Sensors	Myo Armband	200	1	200
Cables	50lb Fishing line	1.00	1	1.00
Battery	1000 mAh 5Volts	1.00	1	1.00
			Total $	350

Table 2 . Comparison of existing commercial myoelectric prosthetics and our design

Name	Type	Weigt	Grip pattern	EMG triggers	Grip control	Cosmetic cover	Adaptive grip	Price
Current design	sEMG	500 g	1	1	EMG	Yes		250 USD
Myoelectrically controlled powered hand prosthetic	sEMG	500 g	1	1	EMG	Yes	NO	300 USD
BeBionic (RSL Steeper)	sEMG	550 g	14	1	Smart-phone app	Yes	Yes	25000-35000 USD
i-Limb (Touch Bionics)	sEMG	460 g	14	1	Smart-phone app	Yes	Yes	20000-100000 USD
Deka Arm (DEKA)	TRI	-	6	4	EMG	No	No	100000 USD
Michelangelo (Ottobock)	sEMG	420 g	6	1	Remote control	Yes	No	100000 USD

Table 3. Results of testing the hand on 7 persons

Motion Gesture	PT1	PT2	PT3	PT4	PT5	PT6	PT7	Average
Open	80/100	90/100	85/100	80/100	95/100	75/100	90/100	85/100
Close	83/100	85/100	91/100	80/100	90/100	70/10	85/100	83/100

PT: Patients

Conclusion

From the results of this study, it is concluded that a simple and cost-effective prosthetic hand can significantly contribute to the development of robotic prosthesis and the results indicate the effectiveness of the proposed method and demonstrate the superiority of the method for amputees because of the improved controllability and perceptibility afforded by the design.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1- Geethanjali, P:Myoelectric control of prosthetic hands: state-of-the-art review. Medical Devices: Evidence and Research 2016:9

2- Balbinot, A., Júnior, A. S., & Favieiro, G. W. . Decoding arm movements by myoelectric signal and artificial neural networks. Intelligent Control and Automation. 2013; 4(01), 87.

3- Raurale, S. A., & Chatur, P. N. . Identification of real-time active hand movements EMG signals for control of prosthesis robotic hand. In Computation of Power, Energy, Information and Communication (ICCPEIC), International Conference on. IEEE 2014; pp.482-487.

4- Brunelli, D., Tadesse, A. M., Vodermayer, B., Nowak, M., & Castellini, C. . Low-cost wearable multichannel surface EMG acquisition for prosthetic hand control. In 2015 6th international workshop on advances in sensors and interfaces. (IWASI) IEEE pp. 94-99.

5- Liarokapis, M. V., Zisimatos, A. G., Bousiou, M. N., & Kyriakopoulos, K. J. . Open-source, low-cost, compliant, modular, underactuated fingers: Towards affordable prostheses for partial hand amputations. In Engineering in Medicine and Biology Society (EMBC), 2014 36th Annual International Conference of the IEEE pp. 2541-2544.

6- Hidalgo, M., Tene, G., & Sanchez. Fuzzy control of a robotic arm using EMG signals. In Industrial Electronics and Control Applications, IEEE 2005. International Conference on pp. 6-pp.

7- Kakoty, N. M., Kaiborta, M., & Hazarika, S. M. . Electromyographic grasp recognition for a five fingered robotic hand. IAES International Journal of Robotics and Automation (IJRA)2012; 2(1), 1-10.

8- Hidalgo M, Tene G, Sanchez A. Fuzzy control of a robotic arm using EMG signals. In: 2005 International Conference on Industrial Electronics and Control Applications Quito; 29 Nov.-2 Dec. 2005; Ecuador 2005: IEEE. pp. 6.

9- Hiyoshi.,.et al,: Development of a Parent Wireless Assistive Interface for Myoelectric Prosthetic Hands for Children. Front. Neurorobot.2018; 12:48

10- Mounika, M. P., Phanisankar, B. S. S., and Manoj, M. : Design and analysis of prosthetic hand with EMG technology in 3-D printing machine. Int. J. Curr. Eng. Technol. 2017; 7, 115–119.

11- Bonini, N., Iyer, N., Kim, D., Mathison, K., & Wellons, L. . Robotic Hand in Motion Using Arduino-Controlled Servos. New Jersey Governor's School of Engineering and Technology, 2014.

12- Ninu, A., Dosen, S., Muceli, S., Rattay, F., Dietl, H., & Farina, Closed-loop control of grasping with a myoelectric hand prosthesis: Which are the relevant feedback variables for force control. 2014 IEEE transactions on neural systems and rehabilitation engineering. 22(5), 1041-1052.

13- Wang, X., Liu, Y., Yang, D., Li, N., Jiang, L., & Liu, Progress in the biomechatronic design and control of a hand prosthesis. In Intelligent Robots and Systems (IROS), 2010 IEEE/RSJ International Conference on pp. 5880-5885.

14- Curline-Wandl, A., and Ali, A. : Single channel myoelectric control of a 3D printed transradial prosthesis. Cogent Eng. 2016; 3:1245541.

Study the Genotoxicity of Aqueous and Alcoholic Extracts of *Adhatoda Vasica* on the Roots of *Allium cepa* L. by RAPD-PCR Technique

Adnan Owayez Jasim[1], Mohammed Mahdi Jawad[1]

[1]Iraq/University of Baghdad – College of Education /Ibn-Al-Haitham-Biology dept.

Abstract

The Molecular technique RAPD-PCR used to detect the genotoxicity of different concentrations of aqueous and alcoholic extracts of *Adhatoda vasica* leaves on onion *Allium cepa* L. roots. Five concentrations (1%, 2%, 3%, 5%, and 7.5%) and (10%, 15%, 20%, 30% and 40%) were adopted for alcoholic and aqueous extracts respectively. Ten arbitrary primers used in this study, only eight showed polymorphic bands in the gel and two primers were neglected because they did not show any polymorphic bands for all samples. The aim of this study is to detect the toxic effect of the extracts on the root of onion. The emergence and disappearance of bundles in the genome of onion plant *Allium cepa* L. Treatment with extracts was studied. The genetic relationship tree was established and the genetic distance calculated based on the results obtained from the gel electrophoresis to clarify the toxic effects of the extracts .The results showed that the concentrations that gave the highest effect and the most toxic for aqueous extract is 40% and for the alcoholic extract is 7.5% which are recommended as effective concentrates if used as a pesticide.

Key words: RAPD,** Adhatoda vasica**, Extracts,** genotoxicity, Allium cepa **L.

Introduction

Acanthaceae a family that composed of many well-known medicinal plants comprises approximately of 3,400 species and 364 genera. The name *Adhatoda vasica* Nees, whose synonym is *Justucia adhatoda* L.[(1)] was selected for experiments on its extracts in this study. *A. vasica* is an evergreen plant. It grows in the form of herbaceous trees distributed all over the world and in various environmental conditions, it lives at an altitude of 1300 m above sea level in the Indian Himalayas [(2,3)].

A. vasica leaves contain a number of chemicals such as alkaloids, flavonoids, turbines, and saponins[(4)]. Alkaloids as Vasicinone. Vasicinol. Adhatodin. Adhatonine. Antisotine and peganine[(5)]. The leaves contain two major alkaloids vasicine and vasicinone[(6)]. Vasicine alkaloid is one of the most effective substances that can be obtained from the leaves of the plant of the seven throat and by 95% of the isolated alkaloids[(7)]. The leaves and flowers of *A. vasica* are used as a medicine because they possess many chemical components such as carbohydrates, protein, phenolsas well as flavanoids and alkaloids[(8)]. *A. vasica* contains saponins and tannins too[(9)], and the roots contain many alkaloids as vasicol, vasicinolone, vasicinone, adhatonine, vasicine and vasicinol[(10)]. Medicinal herbs are a very important source of medicine throughout human history. *A. vasica* is a plant known in ancient Greek medicine as a medicinal plant and used in the medical system for more than 2000 years[(11)], and it is widely used today, indicating that herbs are an increasingly important part of modern medicine. About 25 - 30 percent of the prescribed drugs today contain chemicals derived from plants[(12)]. *A. vasica*, with its various parts of high potential plants, is used for the development of the pharmaceutical and drug industries[(13)]. The plant was used as an anti-asthma and bronchodilator and in the treatment of wounds, ulcers, allergies and pulmonary tuberculosis. Genotoxicity is the sum of DNA damage that causes mutations. When medicinal plants are used to treat diseases, genetic toxicity must be detected. Genotoxicity of *A. vasica* plant extracts. In this study, genotoxicity was detected using the DNA of the onion *Allium cepa* L. roots as a biomarker to confirm the genotoxicity of *A. vasica* extracts.

Materials and Method

Plant sampling

A. vasica leaves were collected during the month of September 2018 from the gardens of the University of Baghdad / Baghdad-Iraq, leaf lengths were 12-27 cm and classified by the herbarium of the college of agriculture - University of Baghdad.

Preparation of extracts

Aqueous extract prepared from 25 g of *A. vasica* leaf powder added to 125 mL of distilled water (in boiling degree) so that the solution is easy to filtrate. Then put the solution on the hotplate stirrer for two hours and filter the solution through 4 pieces of gauze, and centrifuged at 3000 rpm. Place the filtrate in Petri dishes and enter the oven at 40 ° C. Scrape the extracted leaves after drying and store at room temperature until use [14].

To prepare the alcoholic extract, 125 ml of ethyl alcohol at 70% concentration added to 25 grams of dry powdered leaves and transfer the solution in a 50 °C for 24 hours in the shaking water bath. The steps were followed as in the preparation of aqueous extract [15].

Selection of samples and concentrations of extracts

Onions were selected in medium sizes (1.5, 2.2 cm) and 30 g weight , growing well at root lengths (2.5, 3.5 cm) the old roots were removed with dissecting blade and then the onion bulbs transferred to appropriate sized test tubes containing distilled water for 24 hours, then transferred to bottles containing a series of needed concentrations of alcoholic and aqueous extract of *A. vasica* leaves, adopted five concentrations (1%, 2%, 3%, 5%, 7.5%) and (10%, 15%, 20%, 30%, 40%), for alcoholic and aqueous extracts respectively, the onion bulbs still in their concentrations each for 7 days with considering the change of each solution every 24 hours to avoid increasing of concentration by water evaporation. After expiry of the exposure period, roots removed for further studies [16].

DNA extraction

DNA was extracted from onion roots that grown in each concentration solution studied by CTAB (Cetyl Trimethyl Ammonium Bromide) method [17, 18, 19] following the steps recommended by researchers in those references with some modifications.

Measuring the concentration and purity of DNA

The purity and concentration of DNA were measured using the Nano drop spectrophotometer by placing 2µL of each sample in a designated place of the device. The results appeared in private computer software. The DNA purity ranged from (1.7, 1.9) with different DNA concentrations measured in ng/ µl.The quality of the extracted DNA was determined by electrophorsed the samples onto 1% agarose gel .

PCR-RAPD technique

Primers used in RAPD-PCR reaction shown in Table (1) arbitary primers supplied by Intergrati DNA Technology (IDT,USA). and Polymerase chain reaction PCR program shown in Table (2):

Table (1) Primers used in this study

No.	Primer	Primer sequence
1	OPA-1	5́-CAGGCCCTTC-3́
2	OPA-2	5́-TGCCGAGCTG-3́
3	OPA-3	5́-AGTCAGCCAC-3́
4	OPA-4	5́-AATCGGCGTC-3́
5	OPA-5	5́-AGGGGTCTTG-3́
6	OPA-6	5́-GCTCCCTGAC-3́
7	OPA-7	5́-GAAACGGGTG-3́
8	OPA-8	5́-GTGACGTAGG-3́
9	OPA-9	5́-GGGTAACGCC-3́
10	OPA10	5́-GTGATCGCAG-3́

Table (2): PCR program

Cycles No.	Temp. C°	Time	Steps
1	95	4min.	Initial denaturation
40	94	30sec.	Denaturation
	32-40	1min.	Annealing
	72	1min.	Extension
1	72	10	Final extension

Results and Discussion

RAPD –PCR technique

RAPD-PCR technique can be used effectively to detect DNA damage, therefore be used in genotoxicity studies [21, 22]. It can be used without prior knowledge about the genome[23]. It uses random primers with DNA template sequences [24]. It has the ability to detect DNA damage and verify mutations in the DNA when toxic substances are used [25]. Using RAPD-PCR technique in this study revealed the genotoxicity of *A. vasica* leaf extracts as shown in Figure 1 .The emergence or acquisition of bands in gel electrophoresis occurred as a result of changes in the structure of the DNA (fracture, transfers or deletions). As for the deletion or loss of bands, it was due to the reduction of the number of areas of binding with the polymerase [25] or mutation or damage at the site of primer binding to the DNA strand, as well as that loss is as a result of DNA damage, point mutation, chromosomal rearrangement, deletion and addition to the sequences of nitrogenous bases in the DNA strands due to genetotoxicity effect [26]. The primers OPA3, OPA7 were neglected because they did not show any polymorphism in gel bands for all samples.

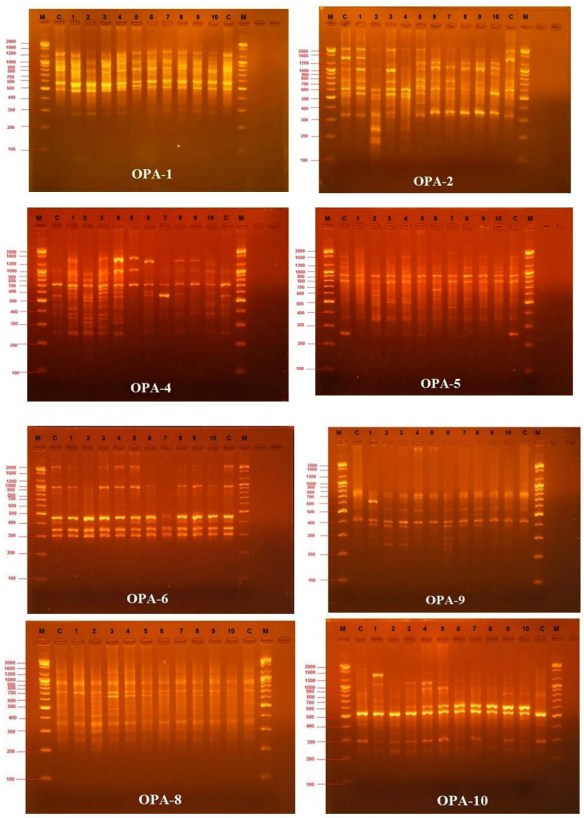


Figure (1) 2% agarose gel electrophoresis of onion roots treated with aqueous and alcoholic extracts of *A. vasica* leaves with molecular marker 100 to 2000bp (from Bioneer / Korea), M–Molecular marker, C-control, 1-1% alcohol, 2-2% alcohol, 3-3% alcohol, 4-5% alcohol, 5-7.5 % alcohol, 6-10% aqueous, 7-15% aqueous, 8-20% aqueous, 9-30% aqueous, 10-40% aqueous.

After determining the purity and concentration of the extracted DNA of the extracts exposed roots, a high concentration of 800 ng/µl was found. Concentrations were reduced to 150 ng/µl for each extract. The results of the RAPD technique showed a variation in the PCR results of samples that exposed to aqueous and alcoholic extracts compared with the control treatment. Table 3 and 4 and Figure 1, show the RAPD-PCR results. It is clear from the results that the eight primers used gave fifty-five bands in control treatment and the molecular weights were between (140- 2109). As for the number of lost and gained bands, it was found that the alcoholic extract was more toxic to the onion roots genome than the aqueous extract.

Table (3) Lost and gained gel bands after PCR in roots of onion plant treated with aqueous extract concentrations.

Primer	Bands control	Band case	10%	15%	20%	30%	40%
OPA-1	7	Gain	5	5	3	3	2
		Loss	5	6	4	4	3
OPA-2	11	Gain	3	3	1	2	4
		Loss	6	8	6	6	7
OPA-4	6	Gain	2	-	2	1	3
		Loss	4	2	4	4	2
OPA-5	6	Gain	4	4	3	1	-
		Loss	3	3	2	1	2
OPA-6	6	Gain	-	-	-	-	-
		Loss	1	3	1	-	1
OPA-8	7	Gain	2	1	4	3	3
		Loss	3	1	2	4	5
OPA-9	4	Gain	4	5	4	2	1
		Loss	3	2	2	1	-
OPA-10	8	Gain	3	4	2	2	2
		Loss	5	5	5	3	3
Total	55	-	53	52	45	37	38

Table (4) Lost and gained gel bands after PCR in roots of onion plant treated with alcoholic extract concentrations.

Primer	Bands control	Band case	1%	2%	3%	5%	7.5%
OPA-1	7	Gain	4	2	3	3	3
		Loss	4	5	3	2	3
OPA-2	11	Gain	1	5	4	2	1
		Loss	2	7	6	7	4
OPA-4	6	Gain	11	7	10	11	2
		Loss	2	1	1	1	4
OPA-5	6	Gain	3	4	3	3	3
		Loss	3	3	2	1	1
OPA-6	6	Gain	-	-	-	-	-
		Loss	2	3	1	1	-
OPA-8	7	Gain	4	6	6	4	2
		Loss	3	3	3	2	2
OPA-9	4	Gain	5	4	6	4	2
		Loss	1	2	3	3	4
FOPA-10	8	Gain	4	2	3	3	4
		Loss	4	5	3	3	4
Total	55	-	53	59	57	50	39

The present study has shown a decrease in viability to death of the roots of *Allium cepa* when treated with different concentrations of both extracts, suggesting that it can be used as a pesticide to weed. Our findings evoke that Low concentrations were cytotoxic to onion roots while high concentrations of 40% and 7.5% of aqueous and alcoholic extracts respectively were prevented cell entry into mitosis. So it was more harmful to the food chain receptors. Consequently, the plant is unable to absorb it for this reason it is recommended to use low concentrations of both extracts as a pesticide[27].

Cluster analysis

UPGMA analysis was used to map the genetic relationship tree[28,29,30] using Jaccard's similarity coefficients the tree results showed groups below(Figure 2).

Group A contains two groups, A1 group included control sample and onion sample exposed to alcoholic extract at 7.5% control (distilled water) and aqueous extract concentrations 20%, 30%, 40%. Group A2 included onion samples exposed to the aqueous extract concentrations of 10% and 15%.

Group B comprised two groups.Group B1 onion samples exposed to the concentration of alcoholic extract 1%, 2%.Either group B2 included samples exposed to alcoholic extract concentrate 3%, 5%.

The results of the genetic relationship tree showed the isolation of the results of aqueous extract from the alcoholic extract, and the isolation of the results of the exposure to control sample and the alcohol concentration of 7.5% were observed clearly.

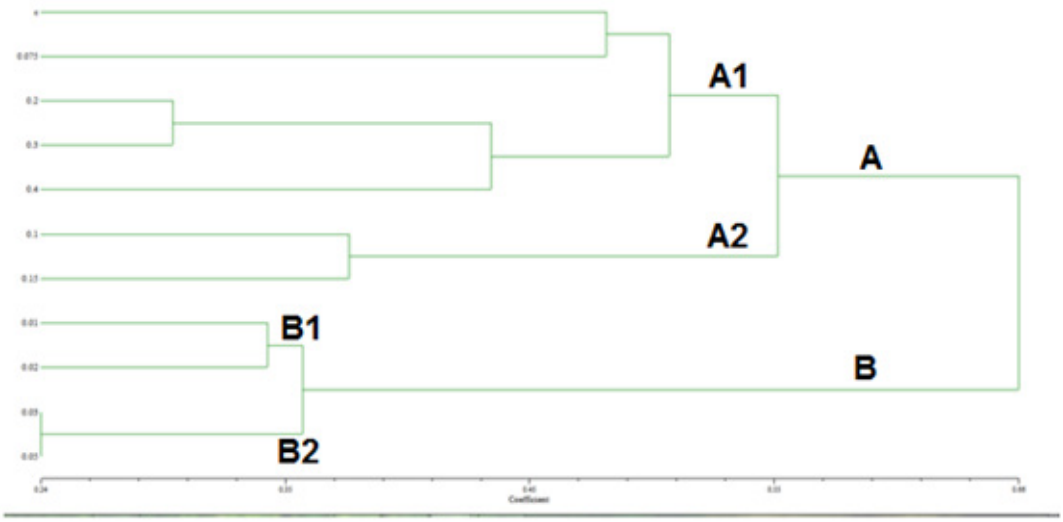


Figure 2: Genetic relationship tree of onion roots treated for aqueous and alcoholic extract concentrations of leaves *A. vasica***.**

The tree showed the isolation of the control sample and the concentration of 7.5% alcohol compared to other samples treated with the extract (aqueous and alcoholic). This indicates that all the concentrations had an effect on the onion genome.

Conclusions

This study revealed that the concentration that gave the highest effect and the most toxic for aqueous extract is 40% and for the alcoholic extract is 7.5% because with these concentrations the plant cells started to die.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Sharma A, Sharma A, Kumar VR, Kumar A. Selectedmedicinal used in traditional medcine. AJPHR. 2015; 3: 30-38.
2. Singhi SK, Patel DJR, Dangi A, Bachle D, Kataria RK. A complete over review on *Adhatoda vasica* a traditional medicinal plant. Plants J. 2016; 5(1): 175 – 180.
3. Chaudhary E, Chauhan R, Chauhan N. Assessment of Bactericidal and Phytochemical Properties of *Adhatoda vasica* various Extracts against Gram Positive and Gram Negative Bacteria. Int J Curr Microbiol App Sci. 2017; 6 (7): 2660-2669.
4. Yusuf M, Khan SA. *Adhatoda vasica* N. leaves extract: phytochemical analysis and antibacterial activity. IJEAS. 2016; 2: 26-32.
5. Claeson UP, Malmfors T, Wikman G, Bruhn JG. *Adhatoda vasica*: a critical review of ethnopharmacological and Toxicological data . J Ethnopharmacol. 2000; 72: 1-20.
6. Kumar KP, Bhowmik D, Tiwari, P, Kharel R. Indian tradition herbs *Adhatoda vasica* and its Medicinal application. J Chem Pharm Res. 2010; 2 (1): 240-245.
7. Keesara BR, Jat RK. Isolation and characterization of Vasicine*Adhatoda vasica* (Adusa). Int J Res Dev Pharm L Sci. 2017; 6(2): 2590-2596.
8. Chaun, WS, Sarangthem K. Phytochemical Constituents of *Justicia Adhatoda* Linn. found in Manipur. JPS. 2014; 3(2): 19- 21.
9. Reddy MP, Shantha IR, Rao VR, Venkateshwarlu. Pharmacognostical and Physicochemical Evaluation on The flowers of *Juaticia adhatoda* L. RJRP. 2015; 7 (2): 73-90.
10. Jain MP, Sharma VK. Phytochemical Investigation of Root of *Ahatoda vasica*. JMPR. 1982; 46 (12): 250 – 255.
11. Sharma A, Kumar A. Acanthace: taxonomy and uses in traditional medicinal system. Word J Pharm Res. 2016; 5:403-412.

12. Prasad R, Lawania, RD, Manvi, Gupta R. Role of Herbs in the Management of Asthma. Pharamacogn Rev. 2009; 3 (4): 247-258.
13. Gangwar AK, Ghosh A. Medicinal uses Pharmacological activity of *Adhatoda vasica*. Inet. J Herb Med. 2014; 2(1): 88-91.
14. Müller J, Helndl A. Drying of Medicinal plants. In: Bogers, R. J; Craker, L. E. and longe, D. (eds). Medicinal and Aromatic plants. Printed in the Netherland. 2006; 237-252.
15. Al-Obaidy SSM. The Effect of some crude Plant Extracts on the cell Division. M.Sc. thesis. The Collage of Science for Woman University of Baghdad. 2004; 146 pp.
16. Olorunfeml DL, Okieimen EA, Ovwemuvwose I. DNA integrity of onion (*Allium cepa* L) root cells exposed to ballast water. studia Unvi. VG, SSV. 2014; 24: 305-309.
17. Doyle JJ, Doyle JL. A rapid DNA isolation for small Quantities of fresh tissue. Phytochem. Bull.1987; 19: 11-15.
18. Doyle JJ, Dickson. Preservation of plant sample for DNA endonuclease analysis. Taxon. 1987; 36: 715-722.
19. Cullings KW. Desing and testing of a plant-specific PCR Primer for ecological and evolutionary studies. Mol Ecol. 1992; 1: 233-240.
20. Stainslav B, Om PR. Protocol: A high-throughput DNA extraction system suitable for conifers. Plant Methods. 2008; 2 (20): 1-6.
21. Atienzar FA, Jha AN. The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: A critical review. Mut Res. 2006; 613: 67-102.
22. Hassan GH, Yassein AA. Cytogenotoxicity evaluation of water Contamination with some texlle azo dyes using RAPD marker and Chromosome aberration of onion (*Allium cepa)* root cell. Egypt J Genrt Cyto. 2014; 43: 39-57.
23. Babu KN, Rrajesh MK, Samaudeen K, Minoo D, Suraby EJ, Anupama K, Ritto P. Randomly amplified polymorphic (DNA) RAPD and derived techniques. Methods mol. Biol., 2014; 1115: 191-209.
24. Makino S, Okada Y, Maruyama T, Kaneko S., Sasakawa C. PCR–based random amplified polymorphic DNA Fingerprinting of Yersinia Pseudotuberculosis and its practical applications. JCM. 1994; 32(1): 65-69.
25. Danylchenko O, Sorochinsky B. Use of RAPD assay for the detection of mutation changes in plant DNA induced by UV-B and Yrays. BMC plant Biol. 2005; 5: 1-2.
26. Yunus MY, Aziz MA; Kadir MA; Daud SK, Rashid. *In vitro* mutagensis of *Etlingera elatior* (Jack) and early detection of mutation using RAPD markers. Turk J Biol. 2013; 37: 716 – 725.
27. Şuţan NA, Popescu A, Mihãescu C, Cristina LS, Marinescu MV. Evaluation of Cytotoxic and Genotoxic Potential of the Fungicide Ridomil in *Allium cepa* L. Anale Stint. univ. Al. l. Cuza Iasi, II a. Bio Veget. 2014; 60 (1): 5-12.
28. Al-Anbari AK, Al-Hadeethi, MA, Jawad, MM. Detection of Genetic Relationships among some specirs belongs to genus *malus* from mid Iraqi regions. Tikrit J. Pure Sci. 2017; 22(2): 27-32.
29. Al-Hadeethi MAH, Aljewari H, Jawad MM, Al-Khesragi TO. Genetic Diversity and Relationships among Verbascum Species in Iragi by RAPD – PCR Technique. Glob J Mol Evol Genom. 2014; 2(2): 62-74.
30. Pinarkara E, Kayis SA, Hakki EE, Sag A (2008). RAPD analysis of seized marijuana (*Cannabis sativa*) in Turkey. EJB. 2008; 12(1): 1-13.

Histological Study of the Effect of Isoxicam on Ovary of Albino Mice Mus Musculus

Ali Khudheyer Obayes[1], Wurood Mohamed Mutar[2], Rasha Hamid Ayub[3], Sahar Abdullah Mohammed[4]

[1]Dep. Biology/ College of Education / University of Samarra/Iraq,
[4]Education of Salahaldin / Dept of Aldor , Tikrit , Iraq

Abstract

Non-Steroidal Anti Inflammatory Drugs (NSAIDs) are the most prescription as therapeutic drugs, used to treat of rheumatic diseases, due to analgesic, antipyretic and anti-inflammatory activity. Isoxicam is a member of NSAIDs group use to stop inflammation, pain associated with arthritis, osteoarthritis, ankylosing and spondylitis. The goal of the present study is to revealed the effect of different doses of Isoxicam on ovaries tissue in mice. Twenty four female mice are randomly divided into control (n = 6) and experimental (n=18) groups. The experimental groups are subdivides into three groups . Each administrated by (0.0714, 0.1428, 0.71428)mg/kg/day for twenty days, respectively; however the control group just injected by distill water. In twenty day, mice were killed and ovaries tissue was prepared for light microscopic examination. All the experimental animals were injected by drug revealed a hyperplasia of germinal cells on the surface of ovary, tongue like projection of primordial oocytes extend to the medulla, multiple oocytes with disarrangement of follicles and deficient of follicular fluid associated with disappearance of oocytes, vacuolation in the cortical layer of the ovary, compressed premature follicle, hypercellularity of follicular cells, degeneration of germinal layer of cortex surface and hyperplasia of primordial oocytes, therefore it is recommended that using of this drug have many side adverse on female fertility.

Key word : *Histological , Isoxicam , ovary , albino mice .*

Introduction

Isoxicam and Meloxicam these drugs are belonging to the oxicam group. Nonsteroidal anti-inflammatory drugs which display a potent analgesic activity and used for treated rheumatoid arthritis, osteoarthritis and other joint diseases. The pharmacological actions of these oxicam are related to inhibition of cyclo-oxygenase (Cox1,2), an enzyme of prostaglandin biosynthesis at the site of inflammation[1]. Prostaglandin, It have been involved as a regulator of several physiological processes in human body such as inflammatory processes in immune response, vasodilator, vasoconstriction, pain perception and fever. Prostaglandin are produced in every tissue of the body (brain, lung, kidney, intestinal digestive system, male and female reproductive system) [(2)].NSAIDs have many adverse effects of liver, dermis, skin eruption and many physiological disorders in rats' testis [3-6].There are an association between use of prescribed NSAIDs and miscarriage [7]. The modern NSAIDs that belong to tenoxicam, lornoxicam, Piroxicam and Isoxicam which belongs to oxicam family prescribed as inhibitors of both types of Cox [8]. A few research consider these drugs a very good antioxidants [9]. The oxicam family acts on inhibit cyclooxygenases COX-1 and 2, It is also inhibits leucocytes activities.

Fertility in females are affected clearly by COX-2. A study on mice female fertility throughout disruption of COX-2, lead to fails in ovulation, fertilization, implantation, and decasualization. These defects were the direct result of the targeted organ-specific COX-2 deficiency[(10)] .

The aim of this study was elevate effect of Isoxicam on the ovary after application of different concentration on mice ovary .

Corresponding author:
Ali Khudheyer Obayes
E-mail: marwan.walady@yahoo.com

Materials and Method

This study was done in medical laboratory department of biology/ Education college/ university of Samarra. Twenty four mature (70 days old) albino Swiss mice Mus musculus Balb/c were employed, weighing (25±3gm) obtained from college of medicine, Tikrit university. They were maintained on 12:12 light: dark bases, and 24 ±2°C with mouse pelleted food and water adlibitum. Female mice were housed in group not bigger than five animals (all from the same experimental group) in plastic cages with metal cover (13*16*30) cm, with wood shavings as bedding material, Twenty four male albino mice were randomly divided into control (n =6) and experimental(n =18) groups. The experimental groups are subdivides into three groups which divided into four groups of mice, each once is injected Intra Peritoneum. with different doses of Isoxicam once daily for 20 days.

Drug administration

Isoxicam ample 200 mg/2ml. Female were injected daily Intra Peritoneum (I.P.) administrated in three doses: Therapeutic dose, over dose1 and over dose2 (0.0714, 0.1428, 0.71428) mg/kg for 20 days respectively [11], and Control group were injected with normal saline 0.9 mg/ L.

Surgical procedure

In twenty one days, the female were anesthetized by chloroform, and the peritoneal cavity was opened through a lower transverse abdominal incision. The ovaries was immediately removed and kept in normal saline. At the end the experimental animals were killed by decapitation.

Histological preparation

The collected tissues Each segments of skin was taken and immersed in 10 % formalin foe 24 hours followed by immersion in graded series of alcohol from 70, 80, 90 and 100 %, then clearing with xylene and embedded in paraffin wax at 60 cº. Blocking of the samples were done and the sectioning were performed using a rotary microtome. The thickness of the sections were 6 micrometer. The tissue sections after application of staining with Hematoxylin and Eosin were mounted on the slides using D.P.X and covered by cover slides [12]. The slides were examined using light microscope and photographed by manipulated camera prepared for this purpose.

Results

Control group

Histological sections of the ovary in the control group show, intact ovarian surface (germinal layer) directly beneath it are tunica albuginea contain numerous primordial follicles, primary oocytes and secondary oocytes, the cells ranged from flat to cuboidal and low columnar cells. Cortex and medulla regions was continuous, Ovarian follicles are various sizes surrounded by theca interna and theca externa as shown. Each follicle contains a single oocyte in the stroma of the cortex. The oocyte within the secondary oocytes was envelop by the zona pellucida and granulosa cells fig (1).

T1 :Therapeutic group

The histological sections of ovarian cortex in this group shows hyperplasia of germinal cells on the surface of ovary, hyper cellularity in follicular cells around follicular cavity, tongue like projection of primordial oocytes extend to the medulla fig (2A), multiple oocytes with disarrangement of follicles and deficient of follicular fluid associated with disappearance of oocytes , hyperplasia of follicular cells primordial oocytes fig (2B).

T2 : Over dose1

This group showed degeneration of germinal cells layer on the surface of cortex with thickening of collagen fibers around premature oocytes, disappearance of oocyte with vacuolation in the cortical layer of the ovary fig (3A). In other sections showed hypercellularity of follicular cells of oocytes in the cortex , degeneration of follicular cells and secondary oocytes with vacuolation of interstitial connective tissue fig (3B). In tertiary follicle increased a multiple layer of follicular cells around oocytes, disappearance of oocytes with decreased amount of follicular fluid and detachment of follicular cells from surrounded connective tissue fig (3C) .other sections of cortex demonstrated compressed premature follicle , degeneration of germinal layer of cortex surface and hyperplasia of primordial oocytes fig (3D).

T3 : Over dose 2

In this group the microscopical examination were show degeneration of germinal epithelium cells of cortex surface, vacuolation in granulosa layers of premature follicle with remnant of oocyte of premature follicle fig (4A). In the surface of cortex showed

disappearance of germinal epithelium ,disarrangement of the other primary follicles with compressed it and partial congestion of blood vessels fig (4B). In other section showed degeneration of follicular cells layer in premature follicle hyper cellularity of connective tissue and vacuolation in between collagen fibers fig (4C). In medulla of ovary showed empty of blood vessels , spaces in between follicular connective tissue, and hypertrophy of the stromal cells fig (4D).

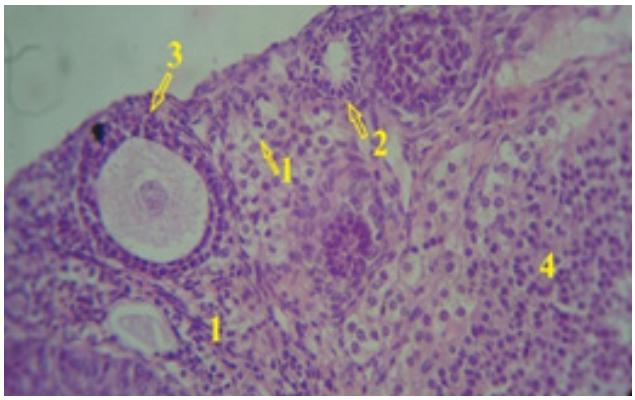


Fig (1) primordial follicles (1), primary oocytes (2) ,secondary oocytes (3) and cortex (4). H&E, 40X .

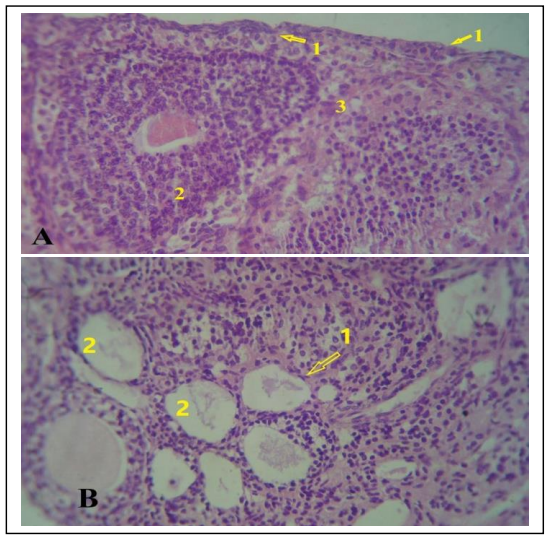


Fig (2): hyperplasia of germinal cells (1), hyper cellularity in follicular cells(2) and tongue like projection of primordial oocytes (3)A. multiple oocytes with disarrangement of follicles with disappearance of oocytes(1), hyperplasia of follicular cells primordial oocytes(2) B. H&E, 40X .

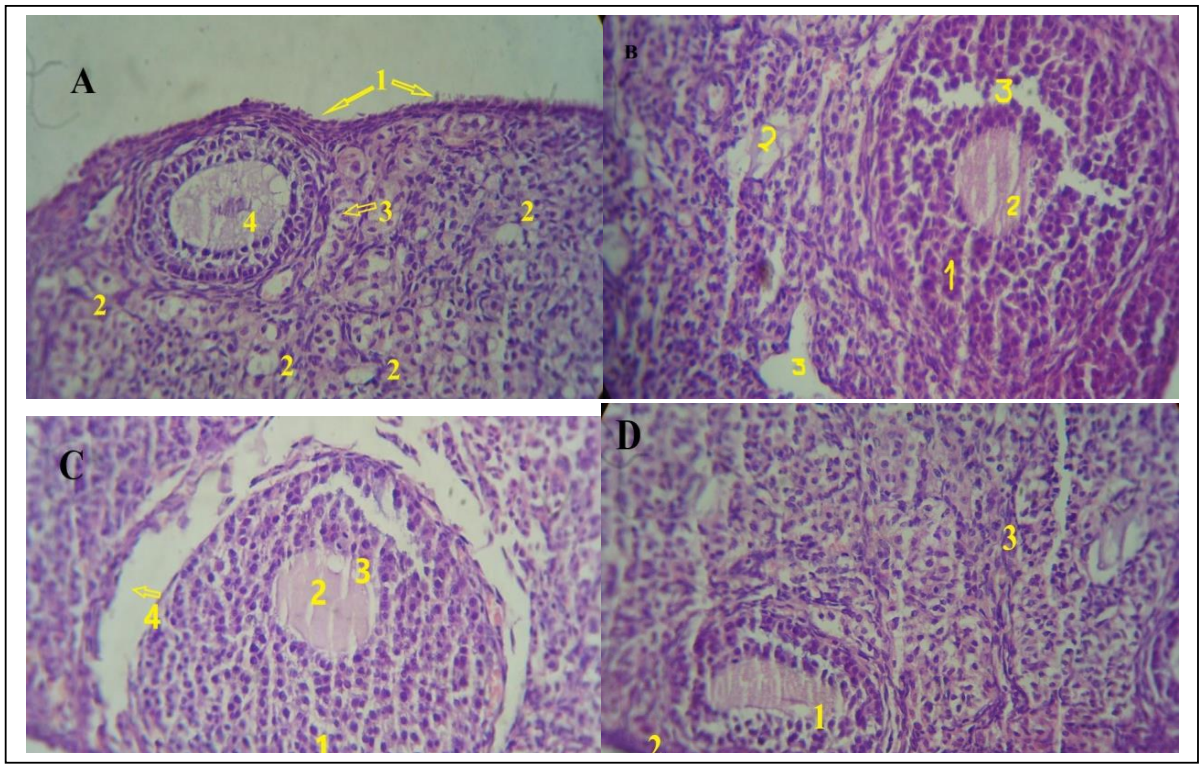


Fig (3) degeneration of germinal cells layer (1), vacuolation in the cortical layer (2), thickening of collagen fibers around premature oocytes (3) disappearance of oocyte (4) A.hypercellularity of follicular cells (1), degeneration of follicular cells (2), vacuolation of interstitial connective tissue (3)B. increased a multiple layer of follicular cells (1), disappearance of oocytes (2), decreased amount of follicular fluid(3) and detachment of follicular cells from surrounded connective tissue(4) C. compressed premature follicle(1), degeneration of germinal layer of cortex surface (2) and hyperplasia of primordial oocytes(3) D.

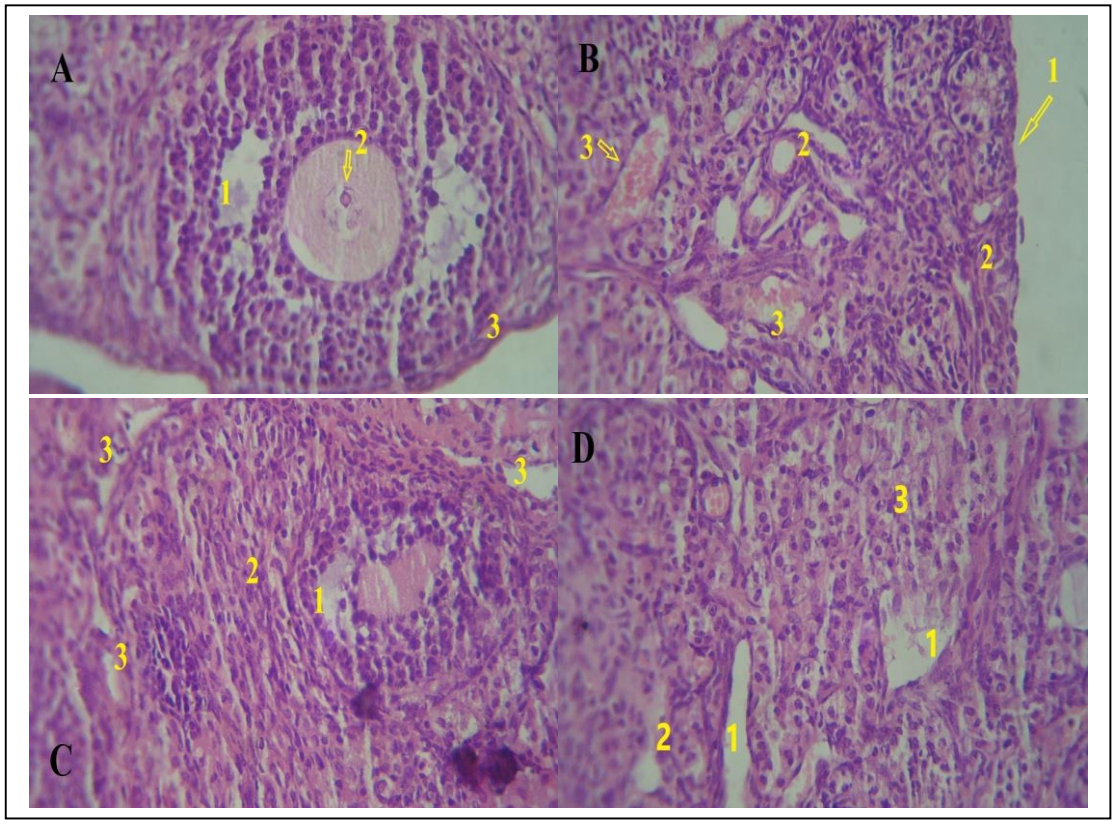


Fig (4): vacuolation in granulosa layers of premature follicle (1), remnant of oocyte of premature follicle(2) and degeneration of germinal epithelium cells of cortex surface(3)A. disappearance of germinal epithelium(1), disarrangement of the other primary follicles with compressed it (2) and partial congestion of blood vessels(3) B. degeneration of follicular cells layer in premature follicle(1), hyper cellularity of connective tissue(2) and vacuolation in between collagen fibers(3) C. In medulla of ovary showed empty of blood vessels(1), spaces in between follicular connective tissue (2) and hypertrophy of the stromal cells(3) D. H&E, 40X.

Discussion

The present study was designed to demonstrate the effect of Isoxicam after application on mice for 21 continuous days, so the Isoxicam was intraperitoneal injection for three different doses The application of this drug demonstrated many histopathological changes in ovaries was affected at different degrees, which means that increasing the concentration lead to severely effect. The female reproductive system is considered to be the most important organ. It characterized by two main functions, synthesis of sex hormones and produce the oocytes [(13)]. Non-steroidal anti-inflammatory drugs (NSAIDs) are the more effectively to reduce pain and inflammation [14].

COX-2 produce prostaglandin which plays a major role in ovulation and fertility. NSAIDS or COX-2 inhibitors which effect on follicle rupture, ovulation, fertilization, luteolysis and parturition when treated rats with indomethacin [15].

Our data was in agreement with [16] who described the effect of ibuprofen at therapeutic dose in mice which induced a histological alteration such as a sequence of events of development and growth of ovarian follicles, increased the number of atretic follicles, Degenerated oocytes of matured follicles with vacuolated stroma. The administration Sodium Metabisulfite on rats which induced histological changes represented by reduced volume of the ovary as well as a decrease in the number of growing follicles, corpus luteum and an increase in the number of atretic follicles. Due to increased lipid peroxidation in the ovarian tissue [17] this study was agreement with our results. The administration of tarragon extract flavonoids reduces cyclooxygenase enzyme and nitric oxide, and thus reduces the amount of prostaglandin which effect on follicle growth [18].Oral administration with indomethacin causes ovulatory dysfunction, represented by the occurred of abnormal follicles at the, with degenerated granulosa cells and reduced follicular fluid of secondary follicle, all these

defect due to COX-2/ prostaglandin synthesis inhibition [19]. Treated rats by atropine sulphate was induced degeneration of granulosa cells and disappearance of antrum the inhibition gonadotrophin release and prostaglandin synthesis[20]. The application of this drug was indicated the insult of Isoxicam in any concentration even therapeutic dose, so our suggestion that this drug must not be used by owner, just used by supervision of the doctors.

Conclusion

The present study revealed that Isoxicam caused a clear histological alterations in ovarian tissue, including hyperplasia of germinal cells on the surface of ovary. hyper cellularity in follicular cells around follicular cavity, tongue like projection of primordial oocytes extend to the medulla, disappearance of oocytes with decreased amount of follicular fluid, vacuolation in granulosa layers of premature follicle with remnant of oocyte of premature follicle. Therefore it is recommended that usage of this drug have harmful side effects on female fertility.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Kumar T , Bairwa, M, Theja D and Srinivas, R. Development and validation of RP-HPLC and ultraviolet spectrophotometric methods of analysis for simultaneous determination of paracetamol and lornoxicam in pharmaceutical dosage forms. Journal of liquid chromatography & related technologies, 2012;35(1):129-140.
2. Modi C, Mody H, Patel G, Dudhatra, G, Kumar A & Avale M. Toxico pathological overview of analgesic anti- inflammatory drug in animals. J. of Applied pharmaceulical Sience, 2012;2(1):149-157.
3. Pandit A, Sachdeva T, & Bafna, P. Drug-induced hepatotoxicity: a review. J Appl Pharm Sci. 2012;2(5): 233-43.
4. Trujillo M, De Barrio M, Rodríguez A, Moreno-Zazo M, Sánchez I, Pelta R, Tornero P. and Herrero T. Piroxicam-induced photodermatitis. Cross-reactivity among oxicams. A case report. Allergologia et immunopathologia, 2001; 29(4):133-136.
5. Özkaya E. Oral mucosal fixed drug eruption: characteristics and differential diagnosis. Journal of the American Academy of Dermatology, 2013;69(2):51-58.
6. Subramanian S.(2009). Diclofenac induced toxic manifestation on adjuvant induced arthritic rats pheripheral and reproductive organ of male wistar rats rattus norvegicus. J. of Toxic & Enviro Health Sci.2009;1(1):12-21.
7. van Gelder M, Roeleveld N, and Nordeng H. Exposure to non-steroidal anti-inflammatory drugs during pregnancy and the risk of selected birth defects: a prospective cohort study. PloS one. 2011;6(7): 22174.
8. Hummdi,L. and Habashi, S. (2012). Histopathological and Ultrastructural Changes in the Duodenal Tissue of Female Mice Treated with Lornoxicam. Middle-East J of Sci Res. 2012;11 (6): 765-776.
9. Demiralay E, and Yılmaz, H. Potentiometric pKa determination of piroxicam and tenoxicam in acetonitrile-water binary mixtures. Süleyman Demirel Üniversitesi Fen Edebiyat Fakültesi Fen Dergisi, 2012;7(1):34-44.
10. Ruan K, Sun S, Herrera V, Ruan, C, and Huynh, L. Cyclooxygenase-2, prostacyclin synthase and fertility. Am J Intgr Med. 2011;1:3-10.
11. Obeys A, and Mahood, A. Histological study of the effect of Piroxicam on testes of albino mice Mus musculus. Journal of university of Anbar for Pure science, 2013;7(2).
12. Bancroft J, and Stevens ,C. Theory and practice of histological techniques .2nd edition .Churchill Livingstone.1982;113-114.
13. Rodrigues J, Navarro P, Zelinski, M, Stouffer R and Xu J. Direct actions of androgens on the survival, growth and secretion of steroids and anti-Müllerian hormone by individual macaque follicles during three-dimensional culture. Human Reproduction, 2015;30(3):664-674.
14. Mazumder S, De R, Debsharma S, Bindu S, Maity P, Sarkar S, Saha S, Siddiqui A, Banerjee

C, Nag S, and Saha D. Indomethacin impairs mitochondrial dynamics by activating the PKCζ–p38–DRP1 pathway and inducing apoptosis in gastric cancer and normal mucosal cells. J of Bio Che. 2019;294(20):8238-8258.

15. Sugimoto, Y., Inazumi, T. and Tsuchiya, S., (2015). Roles of prostaglandin receptors in female reproduction. The Journal of Biochemistry, 2015;157(2):73-80.
16. Sahu C. Developmental toxicity of ibuprofen treated mice. Int J of Pharm Sci. 2009;1:92-102.
17. Rezaee N, Nematollahi Z, Shekarfroush S, & Hoseini E. 2016. Effect of sodium metabisulfite on rat ovary and lipid peroxidation. Iranian J of Toxi. 2016;10(2).
18. Ahmadlo A, Najafian M, Johari H & Kargar H. The effect of tarragon extract on histopathological changes in female rat ovarian tissue. Adv. in Envir Bio. 2012; 6(10):2809-2815.
19. Gaytan M, Bellido C, Morales C, Gonzalez-Padilla M, Sanchez-Criado J and Gaytan F. Immature rats show ovulatory defects similar to those in adult rats lacking prostaglandin and progesterone actions. Repro Bio and Endo. 2004;2(1): 63.
20. MADHU M, Patil S and Saraswati, B. 2014. Effect of Atropine Sulphate on Ovarian Activities in Albino Rats. J of Pharma &Toxic. 20094; (7): 236-245, 2009.

Genotyping of Tumor Necrosis Factor-*α* in Inflammatory Bowel Iraqi Patients

Inas Fadhe[1], Wafaa Sabri Mahood[2]

[1]Post Graduate, [2]Assistant Proff. Dr., College of Education for Pure Sciences- Ibn Al-Haytham, University of Baghdad/Iraq

Abstract

The *TNF-a* gene considered as strong candidate for immune modulator and pro inflammatory cytokine responsible for genetic susceptibility of chronic disease such as the initiation and development of inflammatory bowel disease (IBD).The aim of this study is to investigate the genetic polymorphisms of -1031 in *TNF-a* gene with susceptibility of Iraqi IBD patients

Method: The total number of this study 95 blood samples (75 Iraqi IBD patients and 20 from healthy individuals as a control group). The genetic polymorphisms in *TNF-a*-1031 gene was investigated using restriction fragment length polymorphism (RFLP) and Sanger sequences techniques.

Results: The genotype allele frequency of -1031 polymorphism was significantly higher in CD Iraqi patients (*P*=0.042 , Chi 4.612. OR= 0.72-1.64) ,C allele may be have protective role , whereas the T allele may increase susceptibility to IBD.

In conclusion: The *TNF-a* -1031 gene polymorphisms in promoter region have an important role in the occurrence of inflammatory bowel disease of Iraqi population especially in CD patients although some results didn't give us a significant differences.It's possible that C allele may be have protective role , whereas the T allele may increase susceptibility to IBD.

Key words: *TNF-a -1031 Tumor necrosis factor , inflammatory bowel disease, ulcerative colitis and Crohn 's disease.*

Introduction

IBD include both Crohn's disease (CD) and ulcerative colitis (UC) ,patients with IBD suffer from some common symptoms such as acute diarrhea ,abdominal pain ,fatigue and weight loss [(1)].The location of the two type of inflammation is different ,its affects entire gastrointestinal tract in the CD where the UC affects the ileum and colon [(2)]. Environmental factors ,genetic and immune regulation play a key role in development and progression of IBD which characterized by an irregular immune response of the mucous layer in the intestine to bacterial antigens within the intestinal lumen [(3)]. Regulation of cytokines such as TNF- α and IL-6 play a key role in activation of T helper cells (type 1 and 17) which causes inflammation disease [(4)].TNF-α is the important cytokine for inflammation as it participates in immune response to IBD [(5)].TNF-α characterized with a wide range of inflammatory activity it is usually produce by macrophage and monocyte although there are other types of cells that are produced but in limited quantities [(6)]. Inflamed mucous layer of the intestine in IBD patients have increased gene expression of *TNF-a* gene [(7)] .TNF-α not only stimulates the acute stage of inflammation but plays role for the occurrence apoptosis ,proliferation and differentiation of cells and several immune disorders [(8)].

There is a correlation between the genetic polymorphisms of the encoded gene of TNF-α and the susceptibility to IBD [(9)]. The *TNF*-α - gene have shown several polymorphisms in its four exons. However, most of the common reported polymorphisms are identified in the promoter region of this gene [(10)] . The *TNF*-α promoter region include various a single nucleotides

Corresponding author:
Wafaa Sabri Mahood
email: wafa.sabry@yahoo.com

polymorphisms that have shown a significant association with IBD [11],like *TNF*- α (-308G/A) [12] and *TNF*-α -857 polymorphism [13]. The response to anti TNF- α agents is increased and reduced by the effect of genetic factors of individuals [14].

The aim of this study is to detect the *TNF*-α -1031 gene polymorphisms as its important factor in IBD development and treatment.

Materials and Method

Patients:

Blood samples were obtained from 75 Iraqi patients suffering of IBD who were attending the Gastroenterology and Hepatology Disease Center in Baghdad between September–Desember,2018 .Patients samples were selected after the diagnosis was made by the specialist doctor, in addition to 20 blood samples were collected from apparently healthy individuals. Ethical permission to conduct the research was obtained from this hospital and from all participants in this study.

DNA Extraction

Genomic DNA was isolated from blood sample according to the instructions of ReliaPrep™ Blood gDNA Miniprep System kit (Promega, USA). .

Polymerase chain reaction (PCR)

PCR was done for *TNF*-α-1031 gene amplification, each 20 µl mixture of PCR reaction include 10 µl of master mix , 1 for each primer and 5 distilled water and 3 of genomic DNA. The forward and the reverse primers were 5`-TATGTGATGGACTCACCAGGT-3` and 5`-CCTCTACATGGCCCTGTCTT-3` respectively [15].

PCR amplifications were achieved in Thermal cycler (Applied Biosystem 96). PCR reactions were started through initial denaturation at 95 °C for 5 min followed by 30 cycles of denaturation at 95 °C for 30 s., annealing at 55 °C for 30 s. and extension at 72 °C for 30 s. followed by a final extension at 72 °C for 7 min. then hold at 4 °C.

Restriction Fragment Length Polymorphism (RFLP):

PCR products were digested with restriction enzyme, 1µl from BbsI enzyme (Biolabs, England) was added to 5µl of PCR product for each sample. RFLP was performed using Thermal Cycler (Bio Rad, USA) with the following temperature program: 37 °C for 3 hour, enzyme inactivation at 65°C for 5 minutes followed by 10 min incubation at 4°C to stop the reactions. The restriction enzymes fragments were separated on 2% agarose gel stained with ethidium bromide stain.

Sequencing

PCR products were send to Macrogen Corporation – Korea for Sanger sequencing using (ABI3730XL, automated DNA Sequencer).The results were analyzed using genious software.

Statistical analysis

The Hardy-Weinberg equilibrium was used by the chi-square test to evaluate the frequency of genotypes and correlation of the TNF-α -1031genotypes or alleles between IBD patients and controls group. Calculated Odds ratio (OR) with 95% confidence interval (95% CI) for assessing the correlation strength. All data were analyzed using SPSS (2012). A p value of <0.05 was considered significant [16].

Results and Discussion:

Clinical Characteristic of Patients

The total number of this study was 95 blood samples including 75 blood samples from IBD Iraqi patients in addition to 20 blood samples from healthy individuals as a control group. The IBD patients whose enrolled in this study divided into two groups: the first group 47 UC patients and 28 CD patients. Table 1 refer to the clinical characteristics of IBD patients , the ages where ranged between (19-57) years. High rate of patients less than 50 years (81%) with IBD were found in this study while the older age group or more than 50 years was (19%) with high significant association ($P<0.01$) . This result was agreed with [17] they stated that the peak occurrence of the IBD occurs in the second or third decade of life. High incidence of IBD in females, it was (57%) compared with (43%) in males, significant association ($P<0.05$), was appear depend on patients gender. Statistically significant differences were observed between IBD patients and controls depend on type of disease, patients with UC were (60%) higher than patients with CD (40%), these results consist with other Iraqi study from Erbil city [18], this data may be indicate to that UC has more prevalent than CD in Iraqi population.

Table 1: Clinical Characteristics of IBD Patients

Clinical characteristics	Total No. and Percentage %	Chi-Square
	75(100%)	
Average age		
50 ≥	14(19%)	13.208 **
50 <	61(81%)	
Gender		
Male	32(43%)	5.017 *
Female	43(57%)	
Type of disease		
UC	45(60%)	7.250 **
CD	30(40%)	

Genotyping of *TNF-a* - 1031 gene and Alleles frequency in IBD patients

The genetic polymorphisms in *TNF-a* gene considered as strong candidate for immune modulator and pro inflammatory cytokine responsible for genetic susceptibility of chronic disease and the initiation and expansion of IBD [19].

The genetic polymorphisms in the -1031 (T→C) of *TNF*-α gene promoter (db SNP accession number 1rs1799964) was investigated in Iraqi patients with IBD by PCR-RFLP technique and Sanger sequences to determine the genotypes/allele frequency, at this site there are three genotype of *TNF*-α T-1031C in promoter was found , TT with band sizes 251bp and other small band 13 pb that emerge with primer dimer while TC and CC that have band sizes (251/180/ 71) pb and (180/71) pb respectively Figure 1. The sequences analysis of *TNF-a* – 1031T>C showed in Figure 2 .

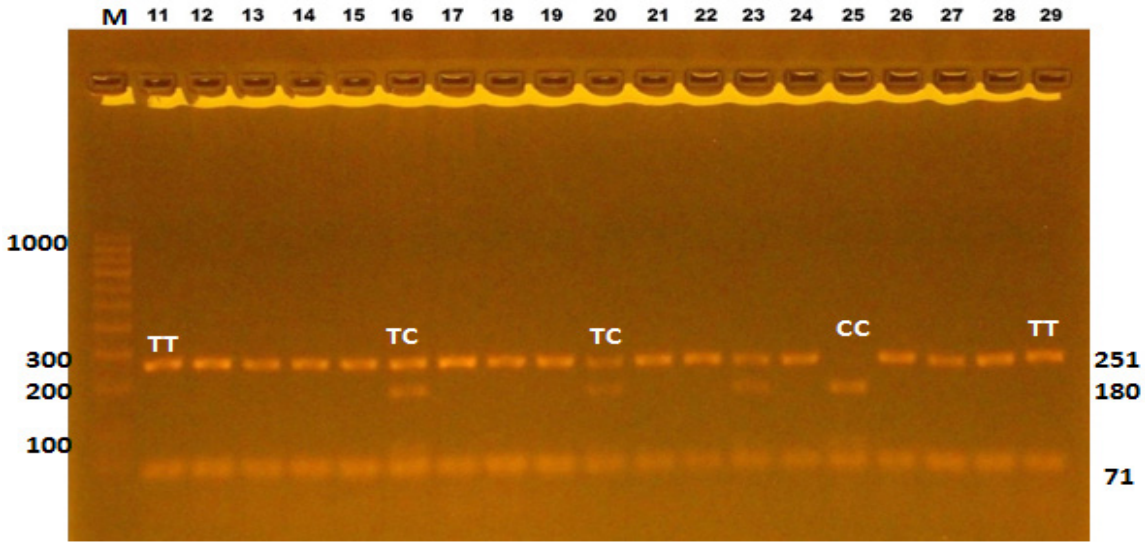


Figure 1: Genetic polymorphism in T -1031 C of TNF-α gene on 2% agarose gel with ethidium bromide dye after digestion with Bbsl enzyme, M : DNA molecular size marker (100bp) , Lane (11) TT genotype (251/13) bp , Lanes (16,20) TC genotype (251/180/ 71) bp and line (25) CC genotype (180,71)bp.

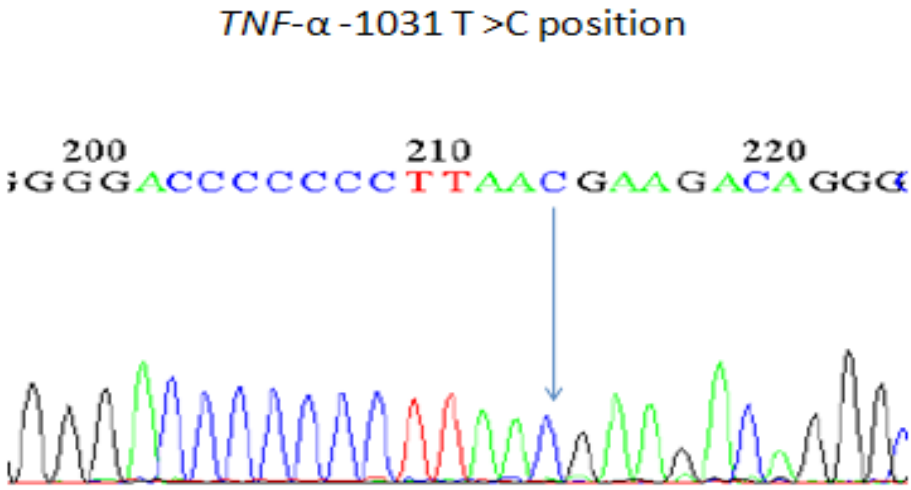


Figure 2 : Sequences analysis of *TNF-a* – 1031T>C using forward primer.

The frequency distribution of genotypes and alleles of *TNF*-α-1031

in IBD patients and control groups are summarized in Tables 2 .The Present results showed 62.7% vs 70 % (*p*=0.061,Chi=3.091 NS,OR=0.377) for homozygous T allele in IBD patients and control group, the homozygous CC genotype was (5.33% vs 5%) in IBD patients and control group , (p=0.872,Chi=0.055 NS ,OR=0.0162) The T>C heterozygous genotype in IBD patients (32%) was higher ratio than control(25%) (p=0.069,Chi=2.983,OR=0.352) the data showed no significant difference was found in correlation to the three genotypes, our results agreed with Iranian population [20].

Alleles frequency for T and C alleles in IBD patients and control group were (0.97, 0.83) and (0.21, 0.17) respectively. The results revealed that there was no significant association regarding genetic polymorphism in *TNF*-α (-1031T/C) polymorphism in IBD patients , statistical analysis indicated that *TNF*-α (-1031T/C) was not a risk factor to IBD.

Table 2: Genotyping of *TNF-a* - 1031 gene in IBD patients

Genotype	Patients IBD N (%) 75(100%)	**Control N (%) 20 (100%)**	***P* -Value**	**Chi-Square**	**OR (CI)**	**EF**	**PF**
TT	47 (62.67%)	14 (70.00)	0.061	3.091 NS	0.377 (0.78-1.49)	0.511	***
TC	24 (32.00%)	5 (25.00)	0.069	2.983 NS	0.352 (0.82-1.55)	***	0.376
CC	4 (5.33%)	1 (5.00)	0.872	0.055 NS	0.0162 (0.69-1.58)	***	0.152
Allele Frequency							
T	0.79	0.83	--	--	--	--	--
C	0.21	0.17	--	--	--	--	--
NS: Non-Significant.							

OR = Odd Ratio CI = Confidence Interval EF = Etiology fraction PF= Preventive fraction

Our study demonstrate that the TT genotype has clearly indicates an etiology for IBD, as it had an OR of 0.377 and Etiologic Fraction (EF) of 0.511 (Table 3), in contrast, the TC and CC genotype have rather preventive role as it had Protective Fraction (PF) of .376 and 0.152 respectively with low OR (0.352 and 0.0162) .With the possibility of C allele may be have protective role , whereas the T allele may increase susceptibility to IBD.

Genetic polymorphisms of *TNF-a* gene in UC patients

Genotype of the *TNF*-α genes (-1031) polymorphisms in 45 UC patients (Table 3) .The data revealed that homozygous TT genotype was found 63.83% vs 70% in UC patients and control group respectively (p=0.067,Chi=3.055 NS,OR=0.362) while homozygous CC genotype in UC patients and control group 6.38% and 5% respectively .Heterozygous T>C genotype in UC patients(29%) was higher than control group (25%) however , the three genotypes in current work didn't give significant differences . The heterozygous T>C genotype in this study similar with [21].

Our results also similar to data by Asghar and his colleagues [22] ,they investigated the possible association between five single nucleotide polymorphism (SNPs) in TNF-a gene promoter polymorphisms including -1031T/C in a Japanese population with endometriosis, their results revealed that –1031 (65.1%) TT , (31.7%) TC, (2.8%) CC, (81.4%)T (18.6%)C, the -1031C polymorphism with no significant difference in the frequency of in the *TNF*-a-1031 gene promoter .

The T allele in promoter region of *TNF*- α gene at -1031 site gave a significant risk for development in Turkish and Iranian IBD especially UC patients and also found that C allele was very low in patients and could have a protective role ,the variation in *TNF*α-1031 T allele may increase the risk for developing UC , single nucleotide polymorphism -1031 T > C could have important effect in pathogencity of IBD that lead to increase TNF-α levels in IBD patients [23]. Increased expression of TNF-α , high serum levels have been documented in intestinal tissues and IBD patients [24].

Table 3: Genotyping of *TNF-α*-1031 gene in UC patients

Genotype	UC Patients N (%) 47(100%)	Control N (%) 20(100%)	*P*-Value	Chi-Square	OR (CI)	EF	PF
TT	30 (63.83)	14 (70.00)	0.067	3.055 NS	0.362 (0.84-1.58)	0.269	***
TC	14 (29.79)	5 (25.00)	0.080	2.741 NS	0.279 (0.78-1.62)	***	0.084
CC	3 (6.38)	1 (5.00)	0.706	0.051 NS	0.0158 (0.83-1.62)	***	0.081
Allele Frequency							
T	0.79	0.83	--	--	--	--	--
C	0.21	0.17	--	--	--	--	--
NS: Non-Significant.							

Conclusion

This paper suggests that *TNF-a* -1031 gene polymorphisms in promoter region has an important role in the occurrence of inflammatory bowel disease of IBD patients in Iraqi population especially in CD patients although some results didn't give us a significant differences. It's possible that C allele may be having protective role, whereas the T allele may increase susceptibility to IBD. Other studies are needed with

large number of IBD patients to support our results.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1- Schoultz I ,Keita A. Cellular and Molecular Therapeutic Targets in Inflammatory Bowel Disease—Focusing on Intestinal Barrier Function. Cells. 2019 ; 8(2): 193.

2- de Lange KM , Barrett JC. Understanding inflammatory bowel disease via immunogenetics. Journal of autoimmunity. 2015 ;64: 91-100.

3- Baumgart D C , Carding S R. Inflammatory bowel disease: cause and immunobiology. The Lancet.2007 ; 369 (9573):1627-1640.

4- Prieto-Pérez R, Almoguera B, Cabaleiro T, Hakonarson H , Abad-Santos F. Association between genetic polymorphisms and response to anti-TNFs in patients with inflammatory bowel disease. International journal of molecular sciences .2016; 17(2):225.

5- Waters J P, Pober JS , Bradley JR. Tumour necrosis factor and cancer. The Journal of pathology. 2013; 230(3):241-248.

6- Wilson AG, Symons JA, McDowell, TL, McDevitt, HO , Duff G W. Effects of a polymorphism in the human tumor necrosis factor α promoter on transcriptional activation. Proceedings of the National Academy of Sciences. 1997; 94(7) :3195-3199.

7- Pierik M, Vermeire S, Steen K V, Joossens S, Claessens G, Vlietinck R , Rutgeerts P. Tumour necrosis factor-α receptor 1 and 2 polymorphisms in inflammatory bowel disease and their association with response to infliximab. Alimentary pharmacology & therapeutics. 2004 ;20(3): 303-310.

8- Aggarwal BB, Gupta SC ,Kim JH. Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey. Blood.2012; 119(3): 651-665.

9- Al-Meghaiseeb E S, Al-Robayan A A, Al-Otaibi M M, Arfin M, Al-Asmari A K. Association of tumor necrosis factor-α and -β gene polymorphisms in inflammatory bowel disease. Journal of inflammation research. 2016; 9, 133–140.

10- Hajeer AH, Hutchinson LV. TNF-α gene polymorphism: clinical and biological implications. Microsc Res Tech. 2000 ;50:216–28.

11- Neuman MG , Nanau RM. Single–nucleotide polymorphisms in inflammatory bowel disease. Transl Res 2012; 160(1): 45–64.

12- Sykora J, Subrt I,Didek P,Schwarz J , Machalva V..Cytokine tumor necrosis factor-alpha A promoter gene polymorphism at position -308 G-->A and pediatric inflammatory bowel disease: implications in ulcerative colitis and Crohn's disease. J Pediater Gastroentrol Nutr. 2006; 42(5): 479-87.

13- Rahbar Kafshboran H, Bonyadi M, Miri H, Haghi M, Nikravesh A, Abdolmohammadi R, Khoshbaten M. Association of TNF-α -857 Polymorphism with Inflammatory Bowel Disease in a Group of Iranian Azeri Individuals. Middle East journal of digestive diseases. 2014; 6(1), 28–31.

14- Dubinsky MC, Mei L, Friedman M, Dhere T, Haritunians T, Hakonarson H, Kim C, Glessner J, Targan SR, McGovern DP , Taylor KD. Genome wide association (GWA) predictors of anti-TNFα therapeutic responsiveness in pediatric inflammatory bowel disease. Inflammatory bowel diseases .2009;16(8):1357-1366.

15- Naderi N, Farnood A, Dadaei T, Habibi M, Balaii H, Firouzi F, Mahban A, Soltani M ,Zali M. Association of tumor necrosis factor alpha gene polymorphisms with inflammatory bowel disease in Iran. Iranian journal of public health .2014;43(5):630.

16- SAS. 2012. Statistical Analysis System, User's Guide. Statistical.Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA .

17- Coppell KJ, Galts CPC, Huizing FY, Norton JK, Gray AR, Schultz K, Hobbs CE, Aluzaite K , Schultz M. Annual Incidence and Phenotypic Presentation of IBD in Southern New Zealand: An 18-Year Epidemiological Analysis. Inflammatory Intestinal Diseases.2018; *3*(1):32-39.

18- Hassan JT , Delmany AS.Epidemiological and clinical characteristics of patients with inflammatory bowel disease in Erbil City Madical Journal of Babylon. . 2018 ;18(4):281285.

19- Al-Mohaya M A, Al-Harthi F, Arfin M, , Al-Asmari A. TNF-α, TNF-β and IL-10 gene polymorphism and association with oral lichen planus risk in Saudi patients. Journal of applied oral science : revista FOB. 2015; 23(3):295–301.

20- Nourian M, Asgharian AM , Asadzadeh Aghdaei H. Lack of association between tumor necrosis factor alpha (TNFα) gene1031C/T polymorphisms and susceptibility to inflammatory bowel disease (IBD). Arak Univ Med Sci J. 2016 ;19:71-79.

21 – Tremelling M, Waller S, Bredin F, Greenfield S , Parkes M. Genetic variants in TNF-α but not DLG5 are associated with inflammatory bowel disease in a large United Kingdom cohort. Inflammatory bowel diseases. 2006 ; 12(3):178-184.

22- Asghar T, Yoshida S, Kennedy S, Negoro K, Zhuo W, Hamana S. The tumor necrosis factor-α promoter -1031C polymorphism is associated with decreased risk of endometriosis in a Japanese population. Human Reproduction . 2004 ;19(11): 2509–2514.

23- Bonyadi M, Abdolmohammadi R, Jahanafrooz Z, Somy MH ,Khoshbaten M. TNF-alpha gene polymorphisms in Iranian Azari Turkish patients with inflammatory bowel diseases. Saudi journal of gastroenterology: official journal of the Saudi Gastroenterology Association. 2014; 20(2): 108.

24- Komatsu M, Kobayashi D, Saito K,Daisuke K, Kaori S, Furuya D, Yagihashi A, Araake H, Tsuji N , Sakamaki S, Niitsu Y , Watanabe N.Tumor necrosis factor-alpha in serum of patients with inflammatory bowel disease as measured by a highly sensitive immuno-PCR. Clin Chem. 2001; 47:1297–1301.

Antibacterial activity of Eruca Sativa Seeds Aqueous Extract Against Human Pathogenic Bacteria

Madeha H. Hussain[4], Aml Hendi Salih[1], Rajaa Hendi Salih[2], Ali S. Hassoon[3]

[1]Assist Prof. Dr. Department of Pharmacy, Medical Institute Tech. Mansour / Middle Techical University, Iraq, [2]Assist Prof. Dr. Department of Biological Science, College of Science / University of Mustansiriyah, Iraq, [3]Assist. Lecturer/Department of Soil and Water Techniques, Al-Musaib Tech. College/ Al-Furat Al-AwsatTech. University,Iraq, [4]Prof. Dr. Department of Pharmacy, Medical Institute Tech. Mansour / Middle Technical University, Iraq

Abstract

This research was achieved with the aim of detecting the Antibacterial efficacy of the watery extract Eruca of Sativa seeds toward human pathogenic bacteria, four Pathogenic species of bacteria(Enterococcus faecalis, staphylococcus aureus, pseudomonas aerogenoso, and Salmonella typhi) . were isolated from different clinical samples at al. Kademia teaching hospital Laboratory. **Results:** The results showed The extract inhibits gram positive bacteria and the mean inhibitory zone for strepto coccus faecalis and S. aureus was 10.4 mm and 14.0 mm respectively. **Conclusion**: The extract had no effect on gram negative bacteria.

Keywords: *Eruca Sativa, seeds aqueous extract, pathogenic Bacteria*

Introduction

Eruca sativa Miller (synonym Eruca vesicaria Rocket) , usually called as Tarmira, Garden salad or Rocket salad or Jarjeer. *E. sativa* is one of the endemic species from the family of Brassicaceae which is cultivated in most cases in countries of Mediterranean sea like Greece, Turkey, and Italy. It is an annual dark-green plant, with height around 20-50 cm, with a taste of spicy pungent [(1)]. Its seed is commonly yellow, but sometimes is reddish yellow or spotted with brown-green spots [(2)].

The seeds have long been used in folk medicine as a lactagogue, aphrodisiac, diuretic, antis -corbutic, antimicrobial, to disintegrate renal calculi and induce vomiting

This used seeds were taken long time in traditional medicine as an aphrodisiac, diuretic, lacagog, anti-microbial, anti-bacterial, to induce vomiting and destroy kidney stones [(3)]. However, Flanders and Abdel-Karim [(4)] indicated that oil of seeds of Eruca sativa contains 93.8% fatty acids (11 ones) including 58.5% erucic acids, 6.7% saturated acids, 28.5 % linoleic acid, 1-2 % linolenic acid and 4.5% oleic acid. Depending to to EL-Gendy [(5)] , oil of Eruca sativa rises count of RBCs and the content of haemoglobin.

S. aureus is one of the Gram-positive bacteria, has a coccus shape and it is usually considered as a member of the microorganisms in the human body frequently. It is a member of the Firmicutes and it is exist on the human skin and in upper tracts of the respiratory system [(6)].

Usually, *Staphylococcus aureus* behaves the commensalism role, atypically colonizing approx. 30% of the population of humans. It can occasionallycause some diseases. Particularlly, it is mostly one of the common pathogens of infective endocarditisand bacteremia . In addition to, it can lead to different infections of soft tissues and skin , especially when mucosal barriers or the skin have been penetrated [(7)].

Pseudomonas aeruginosa have a common capsule, bacili-shaped, Gram-negative bacterium which can leads to occurrence some diseases in humans, animals, and plants. A species of great medical significance.

Corresponding Author:
Ali S. Hassoon
alisalealtaie2015@gmail.com
com.hs.ali@atu.edu.iq

Pseudomonas aeruginosa is an opportunistic pathogen in hospitals for individuals with immunodeficiency. It archetypically infects burns, urinary tract, the airway, wounds, and other infections of the blood.

This is the most frequent cause of the infection of external ear (otitis externa), injuries ,and burns, which is the most common colonizer of the medical devices such as catheters. *P. aeruginosa* can is spreading by the contaminated equipments and isn't carefully cleaned or by the hands of health care workers. It can rarely cause pneumonia acquired from the community [8].

Salmonella typhi, the Gram-negative bacterium also called as *S. enterica* serotype Typhi, which grow in blood and intestines . Typhoid fever or Typhoid is spreading by drinking water or consuming food contaminated with stool of the infected people.

Typhoid is an infectious bacterial disease caused by *S. typhi* which causes differential symptoms. Symptoms may vary from severe to moderate and always start 6-30 d after the exposure to this bacteria [9].

Objective

This study was carried out with the aim of detecting the e Antibacterial efficacy of the watery extract of Eruca Sativa seeds toward some pathogenic bacteria to humans.

Material and Method

a. Bacterial isolates:

Four Pathogenic species of bacteria were isolated from different clinical samples at al. Kademia teaching hospital Laboratory.

The isolates were Gram positive coca, Enterococcus faecalis and staphylococcus aureus (S. aureus). And Gram negative loacilli, pseudomonas aerogenoso, and Salmonella typhi.

Bacteria were isolated and diagnosed according to cultural, morphological and biochemical characters according to [10].

b. subculturing of bacterial isolates

From stock culture, Nutrient broth tubes were inoculated for each bacterial speciesseparately, and the tubes incubated at 37 ° C for 24 h.

d. susceptibility test:

The susceptibility of the seed aqueous extract was determined by disc diffusion method according to [11] ., 0.1 milliliters of each bacterial species containing approximately 17^7 cfu / mL was transferred aseptically and spread on the surface of plates of MullerHinton agar (MH-agar).

Serile discs prepared from the filter paper (6 mm) were impregnated with 20 micro liters of the extract, a standard antibiotic disc gentamicin 40 mg/ml has been used as a positive-control and a disc containing 20 micro liters of sterile distilled water as a negative-control. However, all the done discs have been placed on the surface of MH medium and then the dishes have been incubated at 37 ° C for 24 hr [10] .The test was done in triplicate for each bacterial species.

Results

The average of inhibition Zone diameter was determined after the end of the incubation period.

The extract inhibits gram positive bacteria and the mean inhibition Zone for strepto coccus faecalis and S. aureus was 10.4 mm and 14.0 mm respectively.

The extract had no effect on gram negative bacteria, the results were shown in table 1.

Table (1) Antibacterial efficacy (zone of inhibition) of seeds extract of E. sativa (mm)

Bacterial species	Aurce of isolate	E. sativa extract	Gentamicin positive control	Distilled water negative control
Enterococcus faecalis	Urine	10.4	25:3	-
Staphylococcus aureus	Wound	14.0	22.0	-
Pseudomonas aerogenosa	Burn	-	15.8	-
Salmouella typhi	Stool	-	18.0	-

- : means no inhibition zone.

Discussion

The increasing occurance of resistant bacteria could be due to overuse or misuse of commercially available antimicrobials in addition to side effect and toxicity of some of these antimicrobials, this made scientist try to develop aneffective, alternative, affordable, safer and nontoxic antimicrobials of plant origin.[11, 12] .

Eruca sative seed extract contains several secondary mrtabolites [glucosinolate isothiocyanate, Alkaloids, and theic derivaties, Flavonoid, phenols, Tannins, and erucin which are responsible for their antioxidant and antibacterial activity.[13, 14].

Entero coccus Faecalis and s. Aurus was sensitive to the extract and this may be due to mesh like peptidogly can Layer in their cell wall[15].

While Pseudomonas aerogenosa and Salmonella typhi were resistaut to the extract due to the selective permeability of Lipopoly saccharide membrane to hydrophilic solutes which restrict the entry of the extract [16] . and the extraction method used and type of solvent may affect the result too. [17, 18].

The absence of antimicrobial activity of the extract does not indicale that the plant was inactive or not contain bioactive substances [19].

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

(1) Morales, M.and Janick, J. Arugula: A Promising Specialty Leaf Vegetable. In: Trends In New Crops And New Uses. Janick J, Whipkey A (Eds.). Alexandria, VA: ASHS Press, (2002). pp: 418–423.

(2) Koocheki, A.;Razavi, M. A. and Hesarinejad , M A. Effect of extraction procedures on functional properties of Eruca sativa seed mucilage. Food Biophysics (2012). 7.PP:84-92.

(3) Boulos , L. Medical Plants of North Africa. Text book, single ed. Weiss L, El sevir New York, (1983). P71.

(4) Flander, A.and Abdel - Karim, S.M. . A study of certain drugs used in folk medicine. J Am. Oil Chem . Soc. (1985). 62 (7)pp : 1137 - 1145

(5) El-Gendy, A. M. (Effect of Eruca sativa oil on some haematological and biochemical parameters in male albino rats: A preliminary study. JOURNAL-EGYPTIAN GERMAN SOCIETY OF ZOOLOGY, 2000). 32(A), 255-266.

(6) Masalha, M., Borovok, I., Schreiber, R., Aharonowitz, Y., & Cohen, G. Analysis of Transcription of theStaphylococcus aureus Aerobic Class Ib and Anaerobic Class III Ribonucleotide Reductase Genes in Response to Oxygen. Journal of Bacteriology, (2001). 183(24), 7260-7272.

(7) Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler, V. G. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical microbiology reviews, (2015). 28(3), 603-661.

(8) Balcht A, Smith R . Pseudomonas aeruginosa: Infections and Treatment. Informa Health Care. (1994). pp. 83–84. ISBN 978-0-8247-9210-7.

(9) Murray PR, Rosenthal KS, Pfaller MA Medical Microbiology (6th ed.). Philadelphia, PA: Mosby Elsevier. (2009). p. 307

(10) Cheesbrough M. District Laboratory pratice in tropical countries , part 2 , Cambridge university press Cambridge UK: (2006). pp 137-150 .

(11) Nccls (National Committee for Clinical laboratory Standards). Performance standard for antimicrobial disc susceptibility test . Approved standard , (2000) M2-A7 ,.

(12) Upadhyay A.; Upadhyay I., Johny K and Ven Kitanara Yanan K. Combating pathogenic microorganisms using plant derired antimicrobials . A mini Rev. of mechanistic basis . Biomed Res. Int; (2014). 1-18 .

(13) Rl Zwana H., Mona S., Al-whibi S., Khan F and Soliman A. Chemical composition and antimicrobial activity of Eruca Sativa against pathogenic bacteria and Fungi . J. Animal and Plant Sciences; (2016). 26 (6) :1859-71 .

(14) Gnese A., Sabrina G., Marcello F., Angela A., Placido B and Emanuela M. Eruca saliva seed

extract. A novel natural product able to counteract neuroiflammation . Mol. Med. Reports ; (2018) 17 :6235-44 .

(15) Ramesh K., George V and shiburaj S. Chemical constituents and antibacterial activity of the leaf oil of cinnamomun chemungianum Mohan et Henry . J. Essential oil . Res; (2007). 19 :98-100 .

(16) Gurinder J and Daljit S . Antibacterial and phy to chemical screening of Anethum graveolons, Foeniculum Vulgare . and Trachyspermum ammi . BMC. Compl. Alt . Med; (2009). 9: 30 .

(17) Babu S., Satish S., Mohana O. Raghaverdra M and Raveesha K. Antibacterial evaluation and Phytochemical analysis of some Iranian medicinal plant a gaiust plant pathogenic Xanthomonas pathovars. J. Agricult. Technol.., (2007) 3(2): 307-16 .

(18) Medha H., Emad M., Aml H and Duraid K. Effect of extraction Solvent on determination of active ingredients from Beta vulgaris and their effect on Gram negative and positive bacteria . Biosci . Res. ; (2018) 15 (94) :3998-4004 .

(19) Muthuvelan B and Raja R. Studies on the efficiency of different extraction Procedures on the antimicrobial activity of selected medicinal plants . world . J. Microbiol . Biotechnol. (2008). 1. 24 (12) :2837-42 .

(20) Al-Tawaha A., Al-Karaki G and Massadeh A. Variation of chemical composition, antioxidant and total phenols of essential oil from thyme (origamum syriacum L) . grown under open field conditions and protected soilless condition . Amer-Euras J. of Sustain able Agricult, .(2014), 8(12) :20-6 .

Possible Role of Toxoplasmosis on Gene Sequence Alteration in Patients with Cardiovascular Diseases

Nazar Sh. Mohammed[1], Ammer Abd. Mohammed[2], Salwa S. Muhsin[1]

[1]Department of Medical Laboratory Techniques/ College of health and Medical/ Medical Technical University/ Baghdad/ Iraq (MTU), [2]Department of Community / Medical-Technical Institute/ Baghdad, Middle Technical University /Iraq (MTU)

Abstract

Toxoplasmosis is one of the risky infection my lead to cardiovascular diseases. One hundred therein patients samples were collected with Heart Diseases and there were infected with Toxoplasmosis, in Baghdad educational Hospital from period 1st January 2019 to 1st September 2019. The results show that prevalence of *Toxoplasma gondii* among Hypertensive disease and Myocardiopathy with Heart Diseases. The genotype of *MYLK3* exon to Human cardiac gene by gel electrophoresis, Lane M markers correspond to 500 bp ladder lane 2 of gene band with 600bp. Mutation occurrence in exon 8 the gene sequence CCCAGCCGG were change to CCCATCCGG, and in exon 9 the change occurrence in sequence CTCAAGCCGGAG to CTCAAGGTACAA.

Keywords: *Toxoplasma gondii, Alteration, gene sequence, cardiovascular diseases.*

Introduction

Toxoplasmosis is generally a mild infection with signs of lymphadenopathy, but some patients may develop chorioretinitis whic can progress into blindness(1). Severe neurological disorders may be shown by those immunocompromised patients who are infected with *Toxoplasma gondii* (2). Congenital disorders of newborns may also result from primary toxoplasmosis during pregnancy (3). In humans, heart may be affected by toxoplasmosis (4). with myocarditis (3), myocarditis with pericarditis (21, 22) as well as the acute heart failures (5). Patients with toxoplasmosis who develop myocarditis may present with congestive heart failure, arrhythmias, pericardial effusion and constrictive pericarditis (5). There are few studies on the sero-epidemiology of patients suffering from heart diseases due to toxoplasmosis (6). Our study aimed to determine the association between exposure to *Toxoplasma gondii* and patients with cardiac diseases who attended to Baghdad teaching hospital/ Medical city and the association between seropositive patients and behavioral, demographic as well as the clinical features of patients.

Materials and Method

One hundred therein patients samples were collected with Heart Diseases and there were infected with Toxoplasmosis, in Baghdad educational Hospital from period 1st sJanuary 2019 to 1st September 2019, all these patients were diagnosed with Toxoplasmosis by ELISA, Anti *Toxoplasma* antibodies IgM and IgG. Genetic test were done by conventional PCR Primers used for amplifying *MYLK3* exons

AGCTGGGCGCCTCCTCTTT

CCTGGCATCAGACTGCACC

GTGCCGGGAGACCTGGGTTTGA

CCTGCCCCGTGACTCCTGCTCTAA

Statistical analyzing

Preceded data has been entered to the computer with the use of “Statistical Package of Social Science” Software program, v. 18 (SPSS).

Results

The association between *toxoplasmosis* seroprevalence and the clinical features of patients with cardiac diseases:

Table (1): The prevalence of *Toxoplasma gondii* among Hypertensive disease and Myocardiopathy with Heart Diseases.

Table (1): The association between *toxoplasmosis* seroprevalence and the clinical features of patients with cardiac diseases

Features	Patients' number	Prevalence of toxoplasmosis	
		Number	%
Hypertension			
yes	25	3	12.0
No	15	2	13.3
No	24	9	37.5
Myocardiopathy			
Yes	9	2	22.2
No	38	5	13.1

Mutation occurrence in exon 8 the gene sequence CCCAGCCGG were change to CCCATCCGG, and in exon 9 the change occurrence in sequence CTCAAGCCGGAG to CTCAAGGTACAA of *MYLK3* gene shows in table 2.

To detection the genotype of *MYLK3* exon to Human cardiac gene by gel electrophoresis, Lane M markers correspond to 500 bp ladder lane 2 of gene band with 600bp. Seen in figure 1.

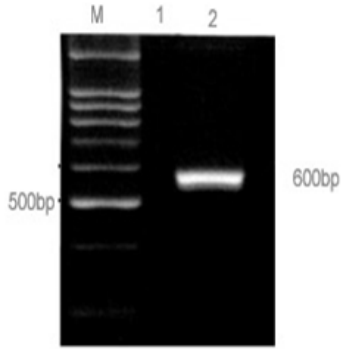


Figure 1: Detection the DNA of (MYLK3 gene) genes Lane M markers correspond to 500 bp ladder (fermintus), lanes 1 & 2and 3 the k13 gene bands with 849 bp.

Table (2) 2 Mutation occurrence in exon 8and exon 9.

Reference	CCCAGCCGG	Variant (c.1915-1G>T) in Exon 8	CCCATCCGG
Reference	CTCAAGCCGGAG	Variant (Exom 9)	CTCAAGGTACAA

Discussion

Toxoplasmosis is one of the risky infection my lead to cardiovascular diseases. Cardiovascular diseases are common conditions in adults that may be in the heart muscles or vessels. *Toxoplasma gondii* due to its presence within the cellular tissue may affect the heart [(1)]. 12% of people with Hypertensive disease are infected with Toxoplasmosis and they are mainly cardiovascular disease. These finding matched with (Flegr, J. *et al*, 2014) who found that 24% of cardiovascular diseases complaining hypertension and they have toxoplasmosis [(7)]. 22.2% of myocardiopathy patients suffer from cardiovascular patients, also they have toxoplasmosis. This report agreed with (England, J. H. *et al*, 2019), who reported that 26% with cardiovascular disease and they complaining myocardiopathy with Toxoplasmosis [(8)]. Cardiovascular disorders are heart conditions, this

phenomenon may be a known source or there or may be a pathogen that causes of these exacerbations or at least be involved in the exacerbation of the condition. Mutation occurrence in exon 8 the gene sequence CCCAGCCGG were change to CCCATCCGG, and in exon 9 the change occurrence in sequence CTCAAGCCGGAG to CTCAAGGTACAA, of *MYLK3* gene, and whole exon sequencing in combination with the segregation analysis of each pedigree with the familial DCM, and determined the read-through mutation i.e (c.2459 A>C; p.*820Sext*19) in the light chain kinase 3 gene (*MYLK3*) of myosin in patients with cardiomyopathy disorders (Tobita, T. et al, 2017), [9]. Cardiomyopathy is the disease of the cardiac muscles. This disease has many clinical features, causes as well as treatments. In most cases, cardiomyopathy causes enlargement, rigidity and thickness of the cardiac muscle. In rare conditions, the ill tissue of the cardiac muscle is replaced with scar tissues. When cardiomyopathy worsens, then the heart becomes weaker. Pumping of blood by the heart becomes less throughout the body and maintaining normal electrical rhythm also becomes less than usual, and it may lead to heart failure or irregular heartbeats known as arrhythmias. Heart valve disorders may also result from the weakened heart [9]. The tropical pulmonary eosinophilias, which have a characteristic of restrictive lung disease and progressive interstitial fibrosis, can lead to PH and then to a course of filarial infection. Intracardiac rupture of *Echinococcus* cyst and *Toxoplasma gondii* may lead to the membrane or secondary cyst embolization of the organs or lungs that are supplied by the systemic circulation. cardiac involvement by parasites must be considered in the differential diagnosis, despite unusual reasons of heart diseases outside the endemic areas, especially in myocardial or pericardial patients of unknown causes. In this study, the present knowledge on the main cardiac diseases caused by the protozoan and metazoan parasites have been updated and summerized, including the heart muscle either directly adversely, (Nunes, M. C. P *et al,* 2007), [10]. The genetic mutation of cardiovascular disease patients associated with toxoplasmosis has been found to exacerbate the pathological condition and alter the gene trajectory of the infected. These findings were in harmony with (Webster, J. P. *et al,* 2013) who reported the behavioral alterations seen in the infected hosts indicate the following: (1) The active manipulation of the parasite's selective benefit; (2) the active manipulation of the host's selective benefit to improve the effects of the infection; (3) The general pathological response of the host of no clear parasite's or host's selective benefit; or, finally, the subtle distinction of the latter grouping known here as (4) the 'by-product pathology' as a result of the accidental toxoplasmosis selected for behavior manipulation in the alternative host species or the stage of the life cycle. In addition, as this perspective study evaluates the applicability of studying *Toxoplasma gondii* in rats (and/or mice), the intermediate hosts as models to help us understand both evolutions and mechanisms that underpin parasite-changed behaviors (ranging from rodent›s predation to some conditions of human schizophrenia), for the first time in this review we have introduced the novel term of '*T. gondii*–rat manipulation–schizophrenia model' [11]. The genetic mutations that occurred in exon 8 and exon 9 on the *MYLK3* gene proved that there was a genetic sequence change in this muscle tissue of the heart due to the involvement of *Toxoplasma gondii* in the exacerbation of the disease. These work agreed with (Ngô, H. M. *et al.*2017) who reported that these data were de convoluted using three biology system topics: "Orbital deconvolution" elucidated upstream, regulatory pathway interconnecting human susceptibile genes, biomarkers, proteomes in addition to transcriptomes. "Cluster deconvolution" showed visual protein-protein interactions included in a process that affects brain function and circuitry, such as leukocyte migration, lipid metabolism and olfaction. Eventually, "disease deconvolution" which identifies correlations between epilepsy and parasite-brain interactions, movement disorders, Alzheimer's as well as cancer. This "reconstruction-deconvolution" provides templates of the progenitor cells' potentiating influences and components affecting human brain parasitism and disorders [12]. Little research has been done on the incidence of people suffering from heart disease may be involved in *Toxoplasma* or may be conducive to these injuries and therefore has been proven that there is a direct relationship with *Toxoplasma* infection and cases of heart disease and this report works for the first time in Iraq.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Rouatbi, M. Amairia, S. and Amdouni, Y. *et al,*. *Toxoplasma gondii* infection and Toxoplasmosis in North Africa a Review, Parasite. 2019; 26: 6.
2. Taibur Rahman, T. and Shekhar, H. U. Understanding the Relationship of Chronic Toxoplasma Gondii Infection and Schizophrenia, J. of Biol. And Micro.2019 DOI: 10.19080/ AIBM.2019.12.555849.
3. Chiebao, D.P. Pena, H. F. and Passarelli, D. *et al,* Congenital Transmission of *Toxoplasma gondii* After Experimental Reinfection With Brazilian Typical Strains in Chronically Infected Sheep*,* Front Vet Sci. 2019; 6: 93.
4. Schlüter D. and Barragan A. Advances and Challenges in Understanding Cerebral Toxoplasmosis, Front Immunol. 2019; 10: 242.
5. Steven E. Lipshultz Yuk M. and Law, *et al,* Cardiomyopathy in Children: Classification and Diagnosis: A Scientific Statement From the American Heart Association, Circulation. 2019;140:e9–e68.
6. Alvarado-Esquivel, C. Jaquez, M. S. and Luis Francisco Sanchez-Anguiano, L. *et al,*Association Between *Toxoplasma gondii* Exposure and Heart Disease: A Case-Control Study, J Clin Med Res. 2016 May; 8(5): 402–409.
7. Flegr, J. Joseph Prandota, J. Sovičková, M. and H. Israili, Toxoplasmosis – A Global Threat. Correlation of Latent Toxoplasmosis with Specific Disease Burden in a Set of 88 Countries, PLoS One. 2014; 9(3): e90203.
8. England, J. H. Bailin, S. S. Gehlhausen, J. R. [2] and Donald H Rubin, D. Toxoplasmosis: The Heart of the Diagnosis, Open Forum Infect Dis. 2019 Jan; 6(1): ofy338.
9. Tobita, T. Seitaro Nomura, S. and Hiroyuki Morita, H. *et al,* Identification of *MYLK3* mutations in familial dilated cardiomyopathy, Sci Rep. 2017; 7: 17495.
10. Nunes, M. C. P. Henriques, M. and Júnior, G. *et al,* Cardiac manifestations of parasitic diseases, Clin. Cardiol. 30, 218–222 (2007).
11. Webster, J. P. Kaushik, M. and Bristow, G. C. *et al, Toxoplasma gondii* infection, from predation to schizophrenia: can animal behaviour help us understand human behaviour? J Exp Biol. 2013 Jan 1; 216(1): 99–112.
12. Ngô, H. M. Zhou, Y. and Lorenzi, H. *et al. Toxoplasma* Modulates Signature Pathways of Human Epilepsy, Neurodegeneration & Cancer, Sci Rep. 2017; 7: 11496.

Toxoplasma Gondii Infection and Toxoplasmosis in North Africa: A Review

Esraa Salman Jasim[1], Noor F. K. Al-Khawaja[2]

[1]Assist. Prof., [2]Lecturer Department of Orthodontics, College of Dentistry, University of Baghdad, Baghdad, Iraq

Abstract

The present study aimed to investigate *Synthesiomyia nudiseta* (van der Wulp, 1883) in the carcasses of dogs and rats in four different localities of Kerbala governorate. during four seasons. The results indicated the appearance of *S. nudiseta* only in the spring and autumn seasons within the urban and agricultural areas, as well as the appearance of this species on the bodies of dogs only without rats. Taxonomy and morphological futures were described. *S. nudiseta* is belonging to the family Muscidae (Order: Diptera) is described as a first time recorded in Iraqi entomofuna. The specimens wear collected from carcasses of dogs and rats at agriculture and urban regions of Kerbala city. The diagnostic characters and mean morphological features were photoed.

Key words: *Diptera, Forensic Entomology, Iraq, Kerbala City, Muscidae, Synthesiomyia.*

Introduction

The species *Synthesiomyia nudiseta* (van der Wulp, 1883) is a wide distributed in the tropical and subtropical regions of the old and new world [(1)]. This fly has contributed to criminal investigations for many countries such as Costa Rica, India, Malaysia, Thailand, and USA [(2-9)] .

This species has been recorded in numerous studies on human bodies in Mexico and Peru [(10)]. It is often recorded on human bodies in urban areas because it feeds on rubbish and decaying vegetables [(11)]. Larval stages were found as well as the presence of adults on the body of a woman in a 13-storey building in Malaysia, the presence of its larvae determined the time of death was nine days [(12)]. Larval stages of this species on human corpses have been studied in detail in Europe, specifically in the Institute of Forensic Medicine as well as the Spanish Legal Institute because of its importance in criminal investigations [(13)].

The larval stage is very important in future of criminal investigations because its presence on human bodies is evidence that the body was in indoor or urban environments [(14)].

In recent years, high temperatures have affected the distribution of these insects, so it is necessary to know the temperature of those areas from which the samples were taken and where they are distributed in the same area, and the association of this species with bodies as well as wounds plays an important role in determining the time of death post-mortem interval (PMI) [(15)]. The aim of current study to investigate the presence of this species on the bodies of animals (dogs, rats) in different localities of Kerbala city.

Materials and Method

The study was during the period from 1/3/2018 to 28/2/2019 within the four seasons (spring, summer, autumn and winter). Two types of vertebrate animals were used in the study (dogs and rats) as shown in Table (1).

The animals used in the experiment were killed in two ways: the first using a sharp knife and the second by a toxic substance (Strychnine sulfate tablet), with dissolving 1 gm of the Strychnine in 5.0 ml of water and administered orally to rats at a dose of 3 ml using a medical syringe, while dogs were given the tablet directly, after killing the animals by the proves kill methods of *S. nudiseta* was investigated after the corpse was placed on the ground after killed immediately to the point of complete decomposition [(16)].

Use three duplicates in each of the above-mentioned transactions. The insects were collected by air net and fly roll trap. The samples were taken to the laboratory and killed by freezing (24h) and so as they mounted by insect pins, the locality and date of collection were recorded. [17,18]

For identification of genus and species were using taxonomical keys: [13] [19-21].

The habitat and morphological features were taken photos by the aid of the digital microscope dino-light with scales of measurements.

Table (1) showed animals with their weights.

Type of animal	Order and family	Scientific name	Wight of carcass kg
Dogs	Carnivora: Canidae	Canis Lupus familiaris (Linnaeus ,1758)	10 - 7
Rats	Rodentia:Muridae	Rattus rattus (Linnaeus ,1758)	0.5-0.35

Results and Discussion

Morphological study

In the current study, the genus Synthesiomyia and species nudiseta were registered as new record to fauna of Iraq. this genus can by recognized from closely genus by: Arista bare; prosternum setulose; scutellum with numerous setae on sides (plate: 1) it has one species (19).

***Synthesiomyia nudiseta* (van der Wulp, 1883)**

Scientific classification

Order: Diptera

Suborder: Brachycera

Family: Muscidae

Subfamily: Azeliinae

Trib: Reinwaardtiini

Genus: ***Synthesiomyia*** Brauer & Bergenstamm, 1893: 9, 110, 178

Species: *S. nudiseta*

Synonyms:*Cyrtoneura nudiseta* Wulp, 1883

Hyadesimyia grisea Gigliotos, 1893

Synthesiomyia brasiliana Brauer & vonBergenstamm, 1893

Discretion of the species: *S. nudiseta* can be diagnostic easily by the antennae and palpi are orange \ yellow in color, the last terminal segment of the abdomen is yellow.

Body: grey to black in color; length about 7-10 mm male and female (Figure 1 A, B)

Head: compound eye bare without hairs, dioptic in male but in female holoptic; antenna, pedicel with clear longitudinal fissure and has hard short bristles, flagellum yellow with arista bear; maxillary palpi yellow (Figure 2 A, B)

Thorax: consists of four longitudinal vittae on mesothorax, sternopleron triangular with four long bristles, the interior is longer and thicker than other.

Legs: black in color, the hind coxa with hairs at inner posterior margin

Wing: hyaline, costa expanded to R_{3+4}, subcostal not striate, apical section of vein M strongly curved forward (Figure 1B)

Abdomen: gray with checker board and differ from sarcophagids flies by the terminal segment is yellow.

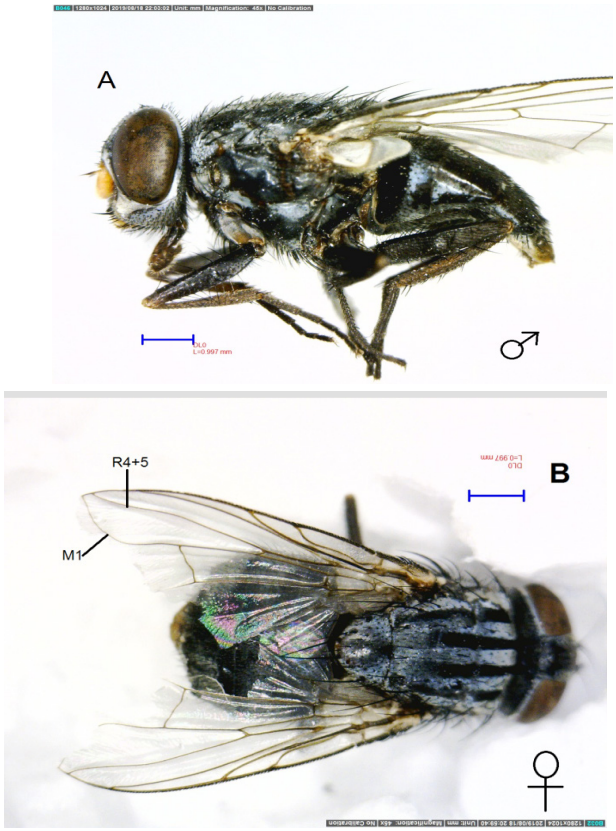


Figure1: (A) Adult male of *S.nudiseta*; and (B) Adult female of *S.nudiseta* with wing M1, R4+5, wing veins.

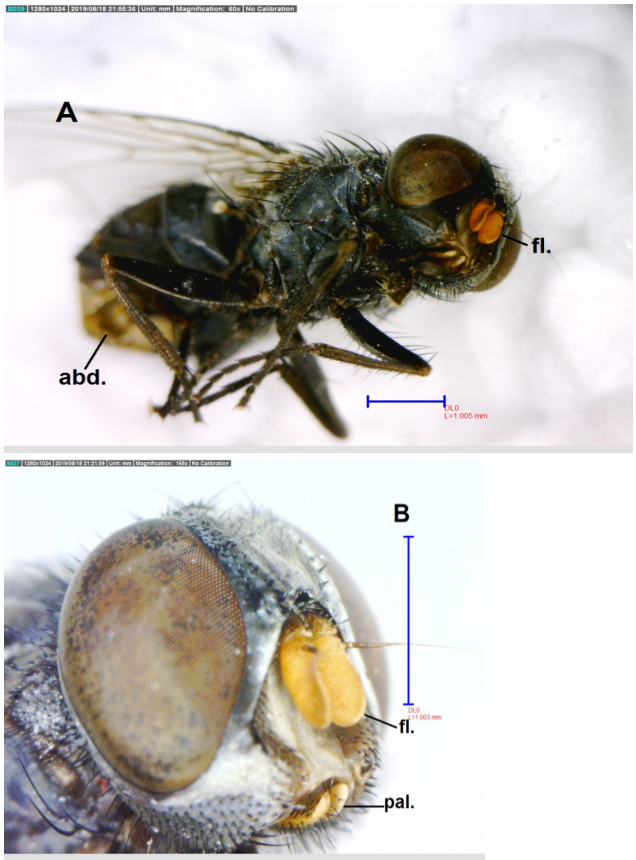


Figure 2: (A)*S.nudiseta*, with orange flagellomere(fl) and tip of abdomen (abd); and (B) head of *S.nudiseta* with flagellomere(fl) and palpe orange.

Environmental Study

The results of the study indicated the effect of attracting *S. mudiseta* insect species according to the type of animal as a number of adults were collected near the bodies of dogs, while no insect was collected near the bodies of mice may be due to the difference in the size of the body [17]. The size of the body has an effect on insect attraction.

As for the method of killing the animal, it has had a great influence in attracting insects. The results of the research indicated that all the insects collected were near the dead bodies with a sharp knife, while no insect was found in the dead bodies using the poison. The amount of blood accompanying the dead body is injurious and does not exist in the case of poisoning [22].

The temperature had an effect on the appearance of *S. mudiseta*. It was observed during the research that the insect is present during the spring and autumn seasons and not fund in winter and summer. The study has indicated that the appropriate thermal range for the appearance of the insect within a temperature range of 20-30 and this is consistent with [23,24].

The results in Figure (3) indicate the geographical area of the environment has had a significant impact on the presence of the insect *S. nudiseta* as it appeared in urban and agricultural areas and does not appear at all in the desert and industrial areas. The puffiness stage is fresh [12][24].

Figure (3) level of appearance that *S. nudiseta* abut, a= autumn; s= summer

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Skidmore, P. The biology of the Muscidae of the world.–Series Entomologica 29: xiv+ 550pp.; Dr. W.

Junk Publishers, Dordrecht. (1985).

2. Jirón-Porras, L. F., Vargas-Arauz, L. G., & Vargas-Alvarado, E. Four muscoid flies (Sarcophagidae and Muscidae) associated with human cadavers in Costa Rica. Cuatro moscas (Sarcophagidae y Muscidae) asociadas con cadáveres humanos en Costa Rica. *Brenesia.*, (1983). (21), 3–5.
3. Lord, W. D., Adkins, T. R., & Catts, E. P. The use of *Synthesiomyia nudesita* [sic](Van Der Wulp)(Diptera: Muscidae) and Calliphora vicina (Robineau-Desvoidy)(Diptera: Calliphoridae) to estimate the time of death of a body buried under a house. *Journal of Agricultural Entomology*, (1992). *9*(4), 227–235.
4. Omar, B., Marwi, M. A., Mansar, A. H., Rahman, M. S., & Oothuman, P. Maggots of *Synthesiomyia nudiseta* (Wulp)(Diptera: Muscidae) as decomposers of corpses found indoor in Malaysia. *Tropical Biomedicine*, (1994). *11*(2), 145–148.
5. Kulshrestha, P., Satpathy, D. K., & Dubey, B. P. Role of *Synthesiomyia nudiseta* (Muscidae) in forensic entomology. In *Proceedings of the Third International Symposium on Advances in Legal Medicine* (1997). (pp. 159–161).
6. Goff, M. L. *A fly for the prosecution: how insect evidence helps solve crimes.* Harvard University Press. (2000).
7. Lee, H. L., Krishnasamy, M., Abdullah, A. G., & Jeffery, J. Review of forensically important entomological specimens in the period of 1972-2002. *Tropical Biomedicine*, (2004). *21*(2), 69–75.
8. Sukontason, K., Narongchai, P., Kanchai, C., Vichairat, K., Sribanditmongkol, P., Bhoopat, T., Bunchu, N. Forensic entomology cases in Thailand: a review of cases from 2000 to 2006. *Parasitology Research*, (2007). *101*(5), 1417.
9. Kumara, T. K., Abu Hassan, A., Che Salmah, M. R., & Bhupinder, S. Larval growth of the muscid fly, *Synthesiomyia nudiseta* (Wulp), a fly of forensic importance, in the indoor fluctuating temperatures of Malaysia. *Tropical Biomedicine*, (2009). *26*(2), 200–205.
10. Huchet, J.-B., & Greenberg, B. Flies, Mochicas and burial practices: a case study from Huaca de la Luna, Peru. *Journal of Archaeological Science*, (2010). *37*(11), 2846–2856.
11. Villet, M. H. Forensic Entomology: The Utility of Arthropods in Legal Investigations. JH Byrd & JL Castner (Eds.): book review. *African Entomology*, (2010). *18*(2), 387.
12. Abdullah, S. R., Ahmad, F. M. S., Bashah, R. M. Z. R. K., Khairul, A. Z., Marwi, M. A., Wahid, S. A., & Omar, B. An occurrence of Synthesiomyia nudiseta (Wulp)(Diptera: Muscidae) from a human corpse in a high-rise building in Malaysia: A case report. *Tropical Biomedicine*, (2012). *29*(1), 107–112.
13. Velásquez, Y., Ivorra, T., Grzywacz, A., Martínez-Sánchez, A., Magaña, C., García-Rojo, A., & Rojo, S. Larval morphology, development and forensic importance of *Synthesiomyia nudiseta* (Diptera: Muscidae) in Europe: a rare species or just overlooked? *Bulletin of Entomological Research*, (2013). *103*(1), 98–110.
14. Pimsler, M. L., Owings, C. G., Sanford, M. R., OConnor, B. M., Teel, P. D., Mohr, R. M., & Tomberlin, J. K. Association of Myianoetus muscarum (Acari: Histiostomatidae) with *Synthesiomyia nudiseta* (Wulp)(Diptera: Muscidae) on human remains. *Journal of Medical Entomology*, (2016). *53*(2), 290–295.
15. Pinto, S. Lo, Giordani, G., Tuccia, F., Ventura, F., & Vanin, S. First records of *Synthesiomyia nudiseta* (Diptera: Muscidae) from forensic cases in Italy. *Forensic Science International*, (2017). *276*, e1–e7.
16. Zaim, M., Seyedi Rashti, M. A., & Saebi, M. E. A guide to medical entomology. *Tehran: University of Tehran Pub*. (2004).
17. Al-Mesbah, H. A study of forensically important Necrophgous Diptera in Kuwait., (July), (2010). 1–136.
18. Al-ashbal, H. N., AL-Essa, R. A.& Abdul-Rassoul, M. S. Survey and diagnostic insect parasites that infect the pupa of house flies Musca domestica Linn . (Diptera : Muscidae) in some region of Karbala. *Journal of Kerbala University*, (2015). *10*(4), 271–289.
19. PONT, A. C. A key to the genera of the Muscoidea (Diptera) recorded from the Fiji Islands. *Bishop Museum Occasional Papers*, (2006). (89), 51–55.
20. Carvalho, C. J. B. de, & Mello-Patiu, C. A. de. Key to the adults of the most common forensic species of Diptera in South America. *Revista Brasileira de Entomologia*, (2008). *52*(3), 390–406.

21. Couri, M. S., Pont, A. C., & Daugeron, C. The Muscidae (Diptera) of New Caledonia. *Zootaxa*, (2010). *2503*(1), 1–61.

22. Alboshabaa, S. H. H., & Al Musawy, H. R. The Taxonomic composition of the forensically important insects in the Rabbit carcasses during two seasons In An-Najaf Province–Iraq. *World J. Pharm. Res*, (2016). *5*, 2068–2077.

23. Aruna Devi, A., Abu Hassan, A., Kumara, T. K., & Che Salmah, M. R. Life table of *Synthesiomyia nudiseta* (Van der Wulp)(Diptera: Muscidae) under uncontrolled laboratory environments–a preliminary study. *Tropical Biomedicine*, (2011). *28*(3), 524–530.

24. Ivorra, T., Grzywacz, A., & Martinez-sanchez, A. (2014). Larval morphology , development and forensic importance of *Synthesiomyia nudiseta* (Diptera : Muscidae) in Europe : a rare species or just overlooked ?, (February 2013).

Study the Cytochrome P450 Gene Expression Changes in Iraqi Patients with Chronic Liver Disease

Areej GH Al-Charak[1], Hamza H Kzar[2]

[1]Lecturer, Department of Physiology and Chemistry, Faculty of Veterinary Medicine, Al-Qasim Green University, Iraq, [2]Lecturer, Department of physiology and Chemistry, Faculty of Veterinary Medicine, Al-Qasim Green University, Iraq

Abstract

The cytochrome P450 is a chemical group of heme-containing proteins speaks to one of the biggest and most practically various superfamilies' found in nature. Chronic liver disease (CLD) is a the process of the liver that involves a process of progressive destruction and regeneration of the liver parenchyma leading to fibrosis and cirrhosis. The aim of this study to assessment the cytochrome P450 gene expression changes of three variants, CYP1A2, CYP2B6, and CYP2E1 in Iraqi patients with chronic liver disease(hepatitis+ alcoholism and hepatitis+ non-alcoholism).CYP1A2, CYP2B6, and CYP2E1 mRNA gene expression were assessed by quantitative real time PCR (qRT-PCR) in 50 cases with CLD and 50 subjects as control. Primers for genes of interest, CYP1A2, CYP2B6, and CYP2E1mRNA and housekeeping gene (GAPDH) were designed by using NCBI tools. Fatty acid synthase (FAS) was estimated by ELISA technique. The results showing statically significant differences in FAS levels (ng/ml) between study groups, HC, HNC, and control (p-value< 0.05). GEF in CYP1A2 gene showing no change between HC and control but there were statistical variations in GEF in HNC and both HC or control. GEF in CYP2B6 and CYP2E1 genes showing highly significant differences between control and both HNC and HC. In conclusion, CYP2B6 and CYP2E1 gene expression were risk factor for progression HC but not HNC through stimulation of increasing levels of FAS.

***Keywords**: chronic liver disease, cytochrome P450, Fatty acid synthase, gene expression.*

Introduction

The cytochrome (P450) is a chemical group of heme-containing proteins speaks to one of the biggest and most practically various superfamilies' found in nature [(1)].The primary capacity of P450s is to encourage the biotransformation of mixes by expansion of practical gatherings reasonable for conjugation and extreme disposal from the life form. The aim of this study to assessment the cytochrome P450 gene expression changes in patients with chronic liver disease [(2)]. In the clinical context, Chronic liver disease (CLD) is a the process of the liver that involves a process of progressive destruction and regeneration of the liver parenchyma leading to fibrosis and cirrhosis [(3)]. Patients with either diagnosed or undiagnosed chronic liver disease occasionally present with an acute deterioration of liver function caused by direct or indirect insults to the liver [(4)]. The expression of CYP 450 enzymes is influenced by endogenous factors, such as genetic polymorphisms, gender, age, and the levels of endocrine hormones [(5)].The expression of CYP45 enzymes is also influenced by exogenous factors such as drugs and environmental chemicals, as well as the physic-pathological conditions [(6)]. CYP1A2 is a member of the cytochrome P450 with a mixed-function of oxidase system, it is involve in the metabolism of xenobiotics in the body and in humans, the CYP1A2 enzyme is encoded by the CYP1A2 gene [(7)]. This quality, CYP2B6, encodes an individual from the cytochrome P450 superfamily of proteins. The cytochrome P450 proteins are mono-oxygenates which catalyze numerous responses engaged with medication digestion and amalgamation

Corresponding author:
Hamza H Kzar
hamza14shukri72@gmail.com

of cholesterol, steroids and different lipids. This protein restricts to the endoplasmic reticulum and its appearance is initiated by phenobarbital. The catalyst is known to use some xenobiotic, for example, the counter malignant growth drugs cyclophosphamide and ifosphamide [8]. CYP2E1 is a layer protein communicated in significant levels in the liver, where it makes about half out of the absolute hepatic cytochrome P450 mRNA[7] and 7% of the hepatic cytochrome P450 protein.[8] The liver is thusly where most medications experience deactivation by CYP2E1, either legitimately or by encouraged discharge from the body.CYP2E1 uses for the most part little, polar particles, including dangerous research facility synthetic substances, for example, dimethyl formamide, aniline, and halogenated hydrocarbons. While these oxidations are frequently of advantage to the body, certain cancer-causing agents and poisons are bio activated by CYP2E1, involving the catalyst in the beginning of hepatotoxicity brought about by specific classes of medications [9]. CYP2E1 also carries out the metabolism of endogenous fatty acids such as the ω-1 hydroxylation of fatty acids such as arachidonic acid, involving it in important signaling pathways that may link it to diabetes and obesity. Thus, it acts as a mono-oxygenase to metabolize arachidonic acid to 19-hydroxyeicosatetraenoic acid (19-HETE) [10]. The aim of this study to assessment the cytochrome P450 gene expression changes of three variants, CYP1A2, CYP2B6, and CYP2E1 in Iraqi patients with chronic liver disease (CLD), (hepatitis +alcoholism and hepatitis +non-alcoholism).

Materials and Method

1- Subjects of the study:

The samples of fresh blood were collected in GIT Centre in Merjan teaching hospital from 50 patients with CLD and 50 healthy subjects as control group. The aged and gender of both groups were matched (p-value> 0.05).

2-Methods: Gene Expression Analysis of CYP1A2, CYP2B6, and CYP2E1

Total RNA was extracted from fresh blood of patents and control by using the TRIzol reagent (USA). The concentration of total RNA was measured by spectrophotometry and the OD260/OD280 ratio was obtained to assess the RNA purity. cDNA synthesis performed by reserve transcript and conducted by PrimeScriptTM RT-PCT reagent Kit (Korea) in a 50 µL reaction mixture following the supplier's instructions. The qRT-PCR was performed by using cDNA as a template in the Exicyclere Real-Time PCR System (Bioneer, Korea) with SYBR green kit as fluorescent dye according to the protocol of manufacture. The PCR conditions were 95 □ for 1min, followed by 40 cycles of 95 □ 15 s, 62 □ 45 s. The primers used in the RT-PCR were designed by NCBI tool and not showing here. The relative gene expression levels were calculated on the basis of $2^{-\Delta\Delta Ct}$. GAPDH was conceder as housekeeping gene as control. The results are presented as fold change of CYPs in patients group compare to control (GEF).

3- FAS levels estimation: Assessed by ELISA depending on Elabscince™ protocol by manufacturer instructions provided with kit.

4-Statistical analysis: Data have been analyzed statistically using SPSS program version 21. Analysis of quantitative data was done using t-test and ANOVA analysis.

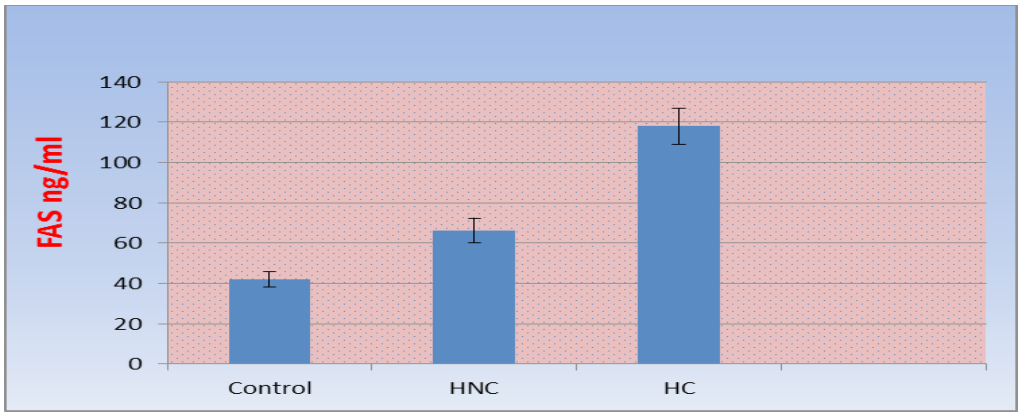
Results

Clinic-pathological characteristics of patients with CLD included in this study are illustrate in table 1:

Table (1): Clinic-pathological characteristics of patients included in this study

Clinic-pathological variables	NO.	%
Total No. of patients	**50**	**100%**
* Age - <30 - ≥30	 22 28	 44 54
* Sex - Male - Female	 26 24	 52 48
*Hepatitis -Hepatitis +Alcoholism (HC) -Hepatitis +Non-Alcoholism (HNC)	 20 30	 40 60

Figure 1, showing the mean±sd of levels of FAS (ng/ml) in patients study group compare to control:



Figure(1): Mean±sd of FAS (ng/ml) in patients groups compare to control

From above figure, there were statically significant differences in FAS levels (ng/ml) between study groups, HC, HNC, and control (p-value<0.05).

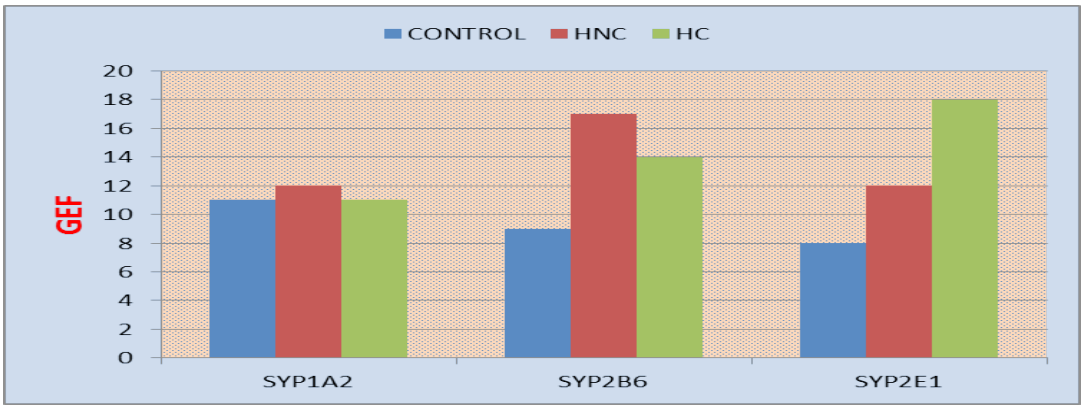
Table 2, showing the levels of FAS (ng/ml) in patients study group depending on age and gender compare to control.

Table (2): **Mean values of FAS levels (ng/ml) for patients compared with control group .**

Clinic-pathological variables	**FAS (mean± SD) ng/ml Age**			**FAS (mean± SD) ng/ml Gender**		
CLD (Hepatitis)	≥ 30	< 30	p-value	**Male**	**Female**	**p-value**
-Hepatitis +Alcoholism (HC) -Hepatitis +Non- Alcoholism (HNC)	123.7±11 67.9±3	113±12 62.3±3	**0.005** **0.048**	123.5±13 68.5±2	122±12 66±1.5	0.084 0.023

From the above table, there were statistical significant differences in levels of FAS ng/ml in HC and HNC subgroups ≥ 30 and < 30 (p-value< 0.05) and this mean that age was risk factor for HC and HNC but the gender in HC was no statistical differences (p-value>0.05) while in HNC the gender was risk factor and increased in males compare to females.

The cytochrome P450 gene expression changes fold of three variants, CYP1A2, CYP2B6, and CYP2E1 in Iraqi patients with chronic liver disease (CLD), (hepatitis +alcoholism and hepatitis +non-alcoholism) compare to control group were illustrated in figure 2:



Figure(2): gene expression fold GEF of CYP1A2, CYP2B6, and CYP2E1 genes in study groups

Discussion

The present study examined the fold of major drug metabolizing P450 (CYP1A2, CYP2B6, and CYP2E1) gene expression fold GEF and FAS levels in 50 Iraqi chronic liver disease (CLD) (hepatitis +alcoholism and hepatitis +non-alcoholism). and compared with 50 normal subjects. FAS is a multi-enzyme protein that catalyzes synthesis of fatty acid . FAS is not a single enzyme but a whole enzymatic system composed of two identical 272 kDa multifunctional polypeptides, in which substrates are handed from one functional domain to the next (11). In the present study, the serum FAS levels of the HC patients were found to be significantly higher in comparison to the HNC and healthy controls, indicating that high concentrations of FAS in serum may result from enzyme secretion by abnormal liver cells. Dorn C et al (2010) were reported that the transcriptional induction of FAS expression in hepatic steatosis is impaired in nonalcoholic steatohepatitis, while hepatic inflammation in the absence of steatosis does not affect FAS expression, suggesting that FAS may be serve as a new diagnostic marker or therapeutic target for the progression of nonalcoholic fatty liver disease (12). Li M et al (2018) were suggested that the accumulation of free fatty acids in hepatocytes induces lipotoxicity, leading to non-alcoholic fatty liver disease (13). GEF in CYP1A2 gene showing no change between HC and control and there were no statistical variations in GEF in HNC and both HC or control, this means that CYP1A2 was not involved in progression of HC and HNC to promoting of chronic liver disease. CYP2B6, and CYP2E1 genes were found help to progression of HC and HNC of chronic liver disease because of highly alteration in GEF in different these groups and this agreement with other studies that using genetic markers as diagnostic agents(14,15). These results suggest a significant role of CYP2B6, and CYP2E1 genes in the regulation of hepatic lipid metabolism via the fatty acid synthesis pathway and FAS, a critical factor for lipid synthesis. In conclusion, The identification of effective gene expression of CYP2B6, and CYP2E1 genes and FAS as molecular and bio markers of HC and HNC could improve the early detection of CLD.

Conflicts of Interest: No conflicts of interest to declare in relation to this work.

Acknowledgment: The authors thanks all the staff of GIT Centre in Merjan teaching hospital for contribution.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1- Nelson DR, Kamataki T, Waxman DJ, Guengerich FP, Estabrook RW, Feyereisen R, Gonzalez FJ, Coon MJ, Gunsalus IC, Gotoh O. The P450 superfamily: update on new sequences, gene mapping, accession numbers, early trivial names of enzymes, and nomenclature. DNA Cell Biol . 1993; 12(2):1–51.

2- Danielson PB. The cytochrome P450 superfamily: biochemistry, evolution and drug metabolism in humans. Curr Drug Metab.2002; 3(1):561–597.

3- Montanini, S; Sinardi, D; Praticò, C; Sinardi, AU; Trimarchi, G . Use of acetylcysteine as the life-saving antidote in Amanita phalloides (death cap) poisoning. Arzneimittel-Forschung. National Institutes of Health. 1999;49 (12): 1044–7.

4- Jalan R, Gines P, Olson JC, Mookerjee R, Moreau R, Garcia-Tsao G, Arroyo V, Kamath P. Acute-on chronic liver failure. J Hepatol. 2012;57(1):1336–1348.

5- Chen H. et al . Expression of P450 and nuclear receptors in normal and end-stage Chinese livers. World J Gastroenterol; 2014;20(26): 8681-8690.

6- Waxman DJ, Holloway MG. Sex differences in the expression of hepatic drug metabolizing enzymes. Mol Pharmacol 2009; 76: 215-228.

7- Nelson DR, Zeldin DC, Hoffman SM, Maltais LJ, Wain HM, Nebert DW. Comparison of cytochrome P450 (CYP) genes from the mouse and human genomes,includingnomenclature.recommendations for genes, pseudogenes and alternative-splice variants. Pharmacogenetics. 2004;14 (1): 1–18.

8- Obach RS, Cox LM, Tremaine LM. Sertraline is metabolized by multiple cytochrome P450 enzymes, monoamine oxidases, and glucuronyl transferases in human: an in vitro study. Drug Metabolism and Disposition. 2005;33 (2): 262–70.

9- Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP . Interindividual variations in human

liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. The Journal of Pharmacology and Experimental Therapeutics. 1994;270 (1): 414–23.

10- Porubsky P, Meneely K, Scott E . Structures of human cytochrome P-450 2E1. Insights into the binding of inhibitors and both small molecular weight and fatty acid substrates. The Journal of Biological Chemistry. 2008;283 (48): 33698–707.

11- Smith S, Witkowski A, Joshi AK . Structural and functional organization of the animal fatty acid synthase. Progress in Lipid Research. 2003;42 (4): 289–317.

12- Dorn C et al (2010). Expression of fatty acid synthase in nonalcoholic fatty liver disease. Int J Clin Exp Pathol.2010; 25 (5):505- 514.

13- Li M et al (2018). Fatty acids promote fatty liver disease via the dysregulation of 3-mercaptopyruvate sulfurtransferase/hydrogen sulfide pathway. Gut. 2018;67(3):2169–2180.

14- Moaed E. Al-Gazally , Alaa S Al-Awad, Hamzah H Kzar. Assessment of antioxidant status in different genotypes/phenotypes at codon 72 of TP53 gene for patients with sporadic colorectal cancer in Babylon province M. International Journal of PharmTech Research, 2016,9(3): 280-286.

15- Moaed E. Al-Gazally, Alaa S. Al-Awad, Hamzah H. Kzar. Evaluating the Superoxide Dismutase-1 status in Wild Type and Mutant at codons 12 and 13 of KRAS gene Spectrum for the Patients with Sporadic Colorectal Cancer. IJPTR,2016, 9(3):272-279.

The Reliability of Orthodontic Treatment, According to the Needs of Patients Using the Dental Aesthetic Index

Esraa Salman Jasim[1], Noor F. K. Al-Khawaja[2]

[1]Assist. Prof., [2]Lecturer, Department of Orthodontics, College of Dentistry, University of Baghdad, Baghdad, Iraq

Abstract

Objectives: Malocclusion was and remains one of the most common problems which affects the psyche and social status of the individual, so the estimation of the malocclusion severity and needs a percentage of orthodontic treatment of Iraqi patients is the aim of this study.

Method: A randomly selected 150 pairs of study models (48 male and 102 female) were involved in this study for patients attending an orthodontic clinic at College of Dentistry/ University of Baghdad seeking for treatment. The DAI scores were collected according to WHO guidelines directly from the study model with a digital caliper, score was calculated using the regression equation of 10 occlusal traits. The dental casts were classified into four groups to determine the treatment needs. SPSS software version 25 was used to analyze the results.

Results: 8.7% of orthodontic treated patients were with normal or mild malocclusion (DAI≤25) and did not need treatment, while 56% of them were handicapped and needed mandatory treatment. In between them, patients with definite and severe malocclusion were 18.7% and 16.6% respectively. Molar deviation (72.6%) represents a high prevalence rate among occlusal traits, while negative overjet (7.3%) is the least one.

Conclusions: Not all patients who are treated in an orthodontic clinic are really in need, and it must be the implementation of treatment need index to determine the treatment priority for patients.

Keywords: *Dental aesthetic index, Treatment need, Study model, Orthodontic patient.*

Introduction

Malocclusion is and remains one of the most common problems which affects the psyche and social status of individual. In addition, a good appearance of the teeth improves acceptance among peer group and increase successful life outcomes in comparison with people of less attractiveness.[1,2,3] One of the major problems in the evaluation of malocclusion is the presence of suitable and objective method to assess and record the severity, complexity, prevalence of malocclusion and the treatment needs.[4] For that this reason many orthodontic indices have been developed since for decades.[5]

William Shaw and colleagues in 1995 classified occlusal indices into five groups;[6] Diagnostic indices, Epidemiologic indices, Orthodontic treatment need indices, Orthodontic Treatment Outcome indices and Orthodontic Treatment Complexity Indices.[7] The Dental Aesthetic Index (DAI) is one of treatment need indices, developed by Cons et al (1986, USA), It has been adopted as a cross-cultural index by the World Health Organization (WHO), a number of researches revealed that the DAI is valid and reliable.[8,9] The index show single score which combine subjective, objective and clinical esthetic factors with a threshold limit (i.e. 31 or higher) to regularize with the needs for orthodontic treatment according to the severity of malocclusion.[10,11] It has been used in several researches within different countries in clinical and epidemiological studies of malocclusion.[12]

The purpose of this study was to estimate severity of malocclusion and needs percentage for orthodontic treatment of Iraqi patients who seek treatment in orthodontic clinic using DAI to know whether they have serious orthodontic problems or not.

Method

Data for this project were retrospectively collected from the orthodontic clinic at College of Dentistry/ University of Baghdad for attending patients who received treatment during the period of September 2017 to January 2019.

A randomly selected 150 pairs of study models (48 male and 102 female) were involved in this study. The patients age ranged from 18 to 25 years with no previous orthodontic treatment, cleft lip and palate, great restorations/crown and/or prosthetic treatment.

The DAI scores were collected according to WHO guidelines directly from the study model with a digital caliper;[13] the index consists of 10 occlusal characteristics including; visible tooth loss, crowding in the incisor region, spacing in the region of incisors, diastema, anterior maxillary misalignment, anterior mandibular misalignment, anterior maxillary overjet, anterior mandibular overjet, vertical anterior open bite and anteroposterior molar relationship.

The core was calculated using the regression equation of 10 occlusal traits: "(visible missing teeth x 6) + (crowding) + (spacing) + (diastema x 3) + (anterior maxillary misalignment) + (anterior mandibular misalignment) + (anterior maxillary overjet x 4) + (anterior mandibular overjet x 4) + (anterior vertical open bite x 4) + (anterioposterior molar relationship x 3) + 13".[14]

Then the dental casts were classified into four groups to determine the treatment needs: Those with score of ≤25 were considered as normal or mild occlusion with little or no need for treatment, scores of 26-30 were defined as malocclusion with elective need for treatment, 31-35 were considered as severe malocclusion with highly desirable need for treatment and if the score

≥36 then it was considered as very severe or disabling malocclusion with mandatory treatment.[15,16]

Statistical analyses. The results were analyzed using SPSS software version 25. The statistics will be:

1) Descriptive statistics: including frequency and percentage.

2) Chi square: To test genders differences.

Calibration: To estimate the reproducibility and validity of the research, 20 dental casts were examined by a specialist orthodontist, and re-examined by the same orthodontist with an interval of 2 weeks to realize intra-examiner accuracy in the employment of the DAI. The intrarater correlation coefficient for repeated examinations was 0.96 ($P < 0.001$), indicating high accuracy.

Results

From the hundreds of patients seeking ~~for~~ treatment, who visited orthodontic clinic at college of dentistry every year, 150 pairs of dental casts for pretreated patients have been used for this study. Chi-Square test showed no significant difference between male and female at a p-value (0.412) as shown in table 1.

The DAI scores were explained in Table 1 and 2, 8.7% of casts with normal or mild malocclusion (DAI≤25), 18.7% of casts with definite malocclusion (DAI 26-30), 16.6% of casts with severe malocclusion (DAI 31-35) and finally 56% of casts with handicap malocclusion (DAI ≥35). The distribution of the total sample according to their DAI scores is illustrated in Table 2, the lowest DAI score recorded was 18 (0.67%) ,while the highest DAI score registered was 91 (0.7%) and the most commonly recorded DAI score was 34 (7.3%).

The distribution of malocclusion components according to the DAI show molar deviation, crowding and anterior maxillary misalignment is the most common among patients which represent 72.6%, 68.7% and 66.7% respectively, and the negative overjet is the lowest among patients (7.3%) as explained in Table 3 and 4.

Table 1 Malocclusion evaluation according to Dental Aesthetic Index. And Gender differences

Dental Aesthetic Index	Female		Male		Total		Malocclusion Severity	Treatment requisite
	n	(%)	n	(%)	n	(%)		
≤25	10	6.6	3	2	13	8.7	Normal or mild occlusion	Little or no need
26-30	18	12	10	6.6	28	18.7	Defined malocclusion	Elective
31-35	17	11.3	8	5.3	25	16.6	Severe malocclusion	Highly desirable
≥35	57	38	27	18	84	56.2	Very severe or disabling malocclusion	Mandatory
Total	102	68	48	32	150	100		
Chi square(X^2) 46.449 P= 0.412								

Table 2 Distribution of the total sample according to their DAI scores

DAI Grade	DAI	n	%	Cumulative %	DAI Grade	DAI	n	%	Cumulative %
Normal or mild occlusion	18	2	1.3	1.3	Very severe or disabling malocclusion	43	3	2	67.3
	20	1	0.7	2		44	3	2	69.3
	22	1	0.7	2.7		45	3	2	71.3
	23	2	1.3	4		46	8	5.3	76.7
	24	3	2	6		47	4	2.7	79.3
	25	4	2.7	8.7		48	2	1.3	80.7
Defined malocclusion	26	5	3.3	12		49	2	1.3	82
	27	2	1.3	13.3		50	3	2	84
	28	4	2.7	16		51	3	2	86
	29	7	4.7	20.7		52	1	0.7	86.7
	30	10	6.7	27.3		53	2	1.3	88
Severe malocclusion	31	3	2	29.3		54	3	2	90
	32	3	2	31.3		57	1	0.7	90.7
	33	2	1.3	32.7		60	2	1.3	92
	34	11	7.3	40		62	1	0.7	92.7
	35	6	4	44		63	3	2	94.7
Very severe or disabling malocclusion	36	8	5.3	49.3		64	2	1.3	96
	37	2	1.3	50.7		65	1	0.7	96.7
	38	7	4.7	55.3		66	1	0.7	97.3
	39	3	2	57.3		70	1	0.7	98
	40	4	2.7	60		74	1	0.7	98.7
	41	4	2.7	62.7		82	1	0.7	99.3
	42	4	2.7	65.3		91	1	0.7	100

Table 3 Distribution of dentition, occlusion and space components according to DAI

DAI components	Present n	%	Absent n	%
Dentitions				
Tooth loss	30	20	120	80
One tooth	20	13.3		
Two tooth	9	6		
Three tooth	1	0.7		
Space				
Crowding	103	68.7	47	31.3
Single jaw	46	30.7		
Both jaw	57	38		
Spacing	54	36	96	64
Single jaw	31	20.7		
Both jaw	23	15.3		
Median Diastema	29	19.3	121	80.7
Anterior Maxillary Misalignment	100	66.7	50	33.3
1-3 mm	66	44		
4-6 mm	32	21.3		
≥7mm	2	1.3		
Anterior Mandibular Misalignment	81	54	69	46
1-3 mm	72	48		
4-6 mm	9	6		
≥7mm				
Occlusion				
Overjet (> 4mm)	67	44.7	83	55.3
Negative overjet	11	7.3	139	92.7
Anterior open bite	21	14	129	86

Table 4 Distribution of molar relationship component according to the DAI

Molar relationship	Present		Absent	
	n	Percent%	N	Percent%
Deviation from the normal molar relationship	109	72.6	41	27.3
Half cusp	62	41.3		
One cusp	47	31.3		

Discussion

The orthodontic clinic at the college of dentistry receives a large number of patients annually from across the country due to lack of specialist orthodontists and the cost of treatment.

In this research, measurements were performed on studying models instead of patients in order to examine a wide variety of patients within a short time,[17] in addition to knowing the patients who visit the orthodontics clinic, the eligible ones for treatment.

The DAI has been supported by WHO due to its simplicity, reliability and wide use in researches,[18] in addition to its high sensitivity in predicting the high proportion of persons requiring orthodontic treatment correctly as well the no-treatment need.[19]

The components of the DAI show the percentage of malocclusion traits and those results can highlight the most commonly presented one as explained in Table 3.

Only one Iraqi study of Kurdish people [20] and few studies of other populations have DAI analysis of malocclusion traits.[9,21,22] Also, because this study is the first study carried out for orthodontic patients who attend the orthodontic department so the results were completely different from previous Iraqi studies. Besides the differences in genetics and race, lead to differences from other populations.

Concerning orthodontic treatment needs, 8.7% of treated patients represent mild or normal occlusion with little or no need for treatment (DAI≤25), while 16.6% (DAI 31-35) of patients have severe malocclusion and 56.2% (DAI≥35) are considered as a being handicapped and in need of mandatory treatment. The female represents 68% of the total sample and that indicate the concern of female about their occlusion and appearance more than male.[23]

Regarding the handicapped malocclusion, the results of our study is close to the results of Poonacha et al. (55%) and Goyal et al. (51%);[9,24] While, it differs from Uzuner et al. (27.8%), Maumela PM and Hlongwa p (41.7%) , Cardoso et al. (39%) and Pop et al. (more than 25%).[21,22,25,26]

But what attracts attention is that orthodontic patients with (DAI≤25) are clearly different from those of other studies which depends on patients who visited the clinic in general. While, our study uses the study models of previously treated patients.

The percentage of each parameter of DAI is explained in Table 3. Where tooth loss was present in 20% of total samples, Crowding in 68.7%, spacing in 36%, median diastema 19.3%, anterior maxillary misalignment 66.7%, anterior mandibular misalignment 54%, overjet (> 4mm) 44.7%, negative overjet 7.3%, anterior openbite 14% and molar deviation from the normal relationship 72.6%.

As we have clarified, the result of each anomaly show much higher rate in comparison with other studies because it harmonious with the fact that the sample was composed entirely of patients referred to an orthodontics clinic with malocclusion, in addition to the racial, genetic, social behaviors, cultural differences and the most common effects are economic and political reasons.

Conclusion

Not all treated patients in the orthodontic clinic are with a true need for treatments, but few of them are with mild malocclusion and no need for treatment; since the therapeutic possibilities are minimally available and poor economic situation for most of the people, the treatment needs should be provided for patients who really deserve.

The DAI is the simplest and easiest index to be used by a general dentist for examination of attending to patients and assessing the severity of their malocclusion to be treated as a priority treatment need.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Preethi K, Nagalakshmi S, Rajkumar B, Vinoth S, Dayanithi D, Bandhari DpK. Assessment of the correlationship of self-perception of dental appearance, smile, and willingness to undergo treatment among school children with dental esthetic index at Thiruchengode. *J Indian Acad Dent Spec Res* [Internet]. 2018;5(1):14. Available from: http://

www.jiadsr.org/text.asp?2018/5/1/14/248279

2. Traebert ESA, Peres MA. Do malocclusions affect the individual's oral health-related quality of life? *Oral Health Prev Dent* [Internet]. 2007;5(1):3–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17366755
3. Ashari A, Mohamed AM. Relationship of the Dental Aesthetic Index to the oral health-related quality of life. Angle Orthod [Internet]. 2016 Mar;86(2):337–42. Available from: http://www.angle.org/doi/10.2319/121014-896.1
4. Gupta A, Shrestha RM. A Review of Orthodontic Indices. Orthod J Nepal [Internet]. 2015 Nov 20;4(2):44–50. Available from: https://www.nepjol.info/index.php/OJN/article/view/13898
5. Hamamci N, Basaran G, Uysal E. Dental Aesthetic Index scores and perception of personal dental appearance among Turkish university students. Eur J Orthod [Internet]. 2009 Apr 1;31(2):168–73. Available from: https://academic.oup.com/ejo/article-lookup/doi/10.1093/ejo/cjn083
6. Shaw WC, Richmond S, O'Brien KD. The use of occlusal indices: a European perspective. Am J Orthod Dentofacial Orthop [Internet]. 1995 Jan;107(1):1–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7817954
7. Borzabadi-Farahani A. An Overview of Selected Orthodontic Treatment Need Indices. In: Principles in Contemporary Orthodontics [Internet]. InTech; 2011. Available from: http://www.intechopen.com/books/principles-in-contemporary-orthodontics/an-overview-of-selected-orthodontic-treatment-need-indices
8. Beglin FM, Firestone AR, Vig KW, Beck FM, Kuthy RA, Wade D. A comparison of the reliability and validity of 3 occlusal indexes of orthodontic treatment need. American Journal of Orthodontics and Dentofacial Orthopedics. 2001 Sep 1;120(3):240-6.
9. Nagalakshmi S, James S, Rahila C, Balachandar K, Satish R. Assessment of malocclusion severity and orthodontic treatment needs in 12-15-year-old school children of Namakkal District, Tamil Nadu, using Dental Aesthetic Index. J Indian Soc Pedod Prev Dent [Internet]. 35(3):188–92. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28762342
10. Wheeler TT, McGorray SP, Yurkiewicz L, Keeling SD, King GJ. Orthodontic treatment demand and need in third and fourth grade schoolchildren. Am J Orthod Dentofac Orthop [Internet]. 1994 Jul;106(1):22–33. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0889540694700176
11. Jenny J, Cons NC. Establishing malocclusion severity levels on the Dental Aesthetic Index (DAI) scale. Aust Dent J [Internet]. 1996 Feb;41(1):43–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8639114
12. Borzabadi-Farahani A. An insight into four orthodontic treatment need indices. Prog Orthod [Internet]. 2011 Nov;12(2):132–42. Available from: http://dx.doi.org/10.1016/j.pio.2011.06.001
13. World Health Organization. Oral health surveys: basic methods, 4th ed. World Health Organization. 1997. http://www.who.int/iris/handle/10665/41905
14. Spalj S, Slaj M, Athanasiou AE, Govorko DK, Slaj M. The unmet orthodontic treatment need of adolescents and influencing factors for not seeking orthodontic therapy. Coll Antropol [Internet]. 2014;38 Suppl 2:173–80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25643546
15. Estioko LJ, Wright FA, Morgan M V. Orthodontic treatment need of secondary schoolchildren in Heidelberg, Victoria: an epidemiologic study using the Dental Aesthetic Index. Community Dent Health [Internet]. 1994 Sep;11(3):147–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7953933
16. Jenny J, Cons NC, Kohout FJ, Jakobsen J. Predicting handicapping malocclusion using the Dental Aesthetic Index (DAI). Int Dent J [Internet]. 1993 Apr;43(2):128–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8320006\
17. Abdul Rahim FS, Mohamed AM, Nor MM, Saub R. Malocclusion and orthodontic treatment need evaluated among subjects with Down syndrome using the Dental Aesthetic Index (DAI). Angle Orthod [Internet] .2014 Jul;84(4):6006. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24417495
18. Onyeaso CO. Orthodontic treatment need of Nigerian outpatients assessed with the Dental Aesthetic Index. Aust Orthod J [Internet]. 2004 May;20(1):19–23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15233583
19. Keay PA, Freer TJ, Basford KE. Orthodontic treatment need and the dental aesthetic index.

Aust Orthod J [Internet]. 1993 Oct;13(1):4–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16429851

20. Al-Huwaizi AF, Ali Rasheed T. Assessment of orthodontic treatment needs of Iraqi Kurdish teenagers using the Dental Aesthetic Index. East Mediterr Health J. 2009; 15:1535-41. Available from:http://www.who.int/iris/handle/10665/117796
21. Maumela PM, Hlongwa P. Application of the dental aesthetic index in the prioritisation of orthodontic service needs. SADJ [Internet]. 2012 Aug;67(7):380–3. Available from:http://www.ncbi.nlm.nih.gov/pubmed/23951797
22. Pop Pop SI, Mártha IK, Csibi R, Păcurar M, Minodora C, Bratu DC. Objective evaluation of orthodontic treatment need with Dental Aesthetic Index. Orv Hetil [Internet].2018 Mar;159(9):352–6. Available from: https://www.akademiai.com/doi/10.1556/650.2018.30943
23. Nanjannawar L, Agrawal JA, Agrawal M. Pattern of malocclusion and treatment need in orthodontic patients: An institution-based study. World J Dent.2012;3(2):136-4.
24. Poonacha K, Deshpande S, Shigli A. Dental aesthetic index: Applicability in Indian population: A retrospective study. J Indian Soc Pedod Prev Dent [Internet]. 2010;28(1):13. Available from: http://www.jisppd.com/text.asp?2010/28/1/13/60483
25. Uzuner FD, Kaygısız E, Taner L, Güngör K, Gençtürk Z. Angle's classification versus Dental Aesthetic Index in evaluation of malocclusion among Turkish orthodontic patients. J Dent App. 2015;2(3):168-73.
26. Cardoso CF, Drummond AF, Lages EMB, Pretti H, Ferreira EF, Abreu MHNG. The Dental Aesthetic Index and dental health component of the Index of Orthodontic Treatment Need as tools in epidemiological studies. Int J Environ Res Public Health [Internet]. 2011;8(8):3277–86. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21909306

Study the Protective Role of Arabic Gum Extract on Some Physiological and Histological Criteria for Liver of Male Rabbit Treated with Atrazine

Noor Rahi AL-Shimmari[1], Rasha Abdulameer Jawad[1]

[1]Department of Biology, College of Education for pure Sciences, Karbala University, Iraq

Abstract

The study involved fifteen health adult male rabbit white (*Lepus arcticus*), weight ranged (1480-1550) kg. and age between 6 to 8 month, divided to three groups the first group treated 10 ml of atrazine orally for five rabbits daily for two weeks and second group is treated with aqueous extract of Arabic gum (AG) (*Acacia Senegal*)30% and atrazine 10 ml between one day and another for two weeks and third group was the control group given 10 a normal saline. In the search the levels of hepatic enzyme were presented (AST) Aspartate transaminase, (ALT) Alanine transaminase and (ALP) Alkaline phosphate, also the rate of diameters of liver sinuses, the diameters of central veins and hepatocellular diameter, and found through this study:

❖ The presence of a significant increase (P≤0.05) in the levels of hepatic enzyme (AST), (ALT) and (ALP) and rate of diameters each of liver sinuses, central veins, and hepatic cells in atrazine group compared with the control group.

❖ The presence of significant decrease (P≤0.05) in levels of hepatic enzyme (AST) and (ALT) in the group treated with aqueous extract of *A*G in 30% concentration and 10 ml of atrazine compared with control group, and did not occur significant differences (P≥0.05) in the level of (ALP) and in rate of diameters each of liver sinuses ,central veins , and hepatic cells in aqueous extract of *Acacia Senegal* and atrazine group compared with the control group

Keyword: *Atrazine, Acacia Senegal, AST, ALT, ALP, Hepatocytes.*

Introduction

Medicinal plants containing active substances used for therapeutic purposes or in the pharmaceutical industry and that the natural products of these plants are an effective source to discover vital effectiveness like anticancer, antioxidant [(1)]. antibacterial and antiparasitic [(2)]. Where these plants are a food source on the one hand and medication against various diseases on the other due to the contents of its parts of chemical compounds of great interest and importance because of therapeutic activity of humans[(3)]. where used as catalysts for growth[(4)].the orientation for medicinal plant treatment is justified and taking chemically manufactured drugs has been shown to have serious side effects that may appear over time and cumulatively[(5)].

AG is a substance collected from the secretion of the stems and branched of *Acacia Senegal* tree, it consists mainly of large molecules carbohydrates, proteins, minerals and amino acid [(6)]. AG has a high molecular weight as polysaccharide it contains galactose, rhamnose, glucuronic acid and arabinose also calcium, potassium and sodium salts. It also contains 1.5%-2.6% protein, 0.22%-0.39% nitrogen and amino acid mainly like hydroxyproline, serine, proline, and aspartic acid, it has high solubility in water but does not dissolve in alcohol [(7)]. And has many uses in the industrial section such as textiles and cosmetics, and pharmaceutical and nutritional uses where in the pharmaceutical field as a pharmaceutical compound because of its antioxidant activity and positive effect in treatment of urinary tract, cardiovascular and digestive system also improves the liver, kidney & heart tissue [(7)].

Atrazine effects on the body's various organs, including the liver, which is the main organ for detoxification of the body. Research has shown a decrease in the accumulation of hepatic glycogen and the presence of early symptoms of liver cytotoxicity, this is due to the toxic effects of atrazine on the liver that inhibit the activity of major enzymes such as hexokinase, glycogen synthase and glucokinase[8], this explains the low body weight has been observed to lead to decrease in glycogen and increase fat in liver[9]. In addition, bile duct hypertrophy and renal tube necrosis have been observed [10]. It has also been found to lead to changes in insulin resistance and disturbance in fat digestion [11]. Atrazine also attacks fats, proteins and DNA molecules, causing metabolic changes in severe cases, it can lead to cell death and lead to oxidative stress and emergence and development of many diseases such as atherosclerosis, cancer, psoriasis, Alzheimer's, high blood pressure, heart disease, liver, kidney and brain [12].

Materials and Method

AG samples were collected from Babylon governorate in October 2018, Gum Arabic powder was moistened with water by 1:5 where 50 of gum Arabic was mixed with 250 ml of distilled water [13]. a horizontal shaker GFL type, 3015 modules for 30 minutes and the sample was left static and then filtered using filter paper three times, after that centrifuge was used 3000 rpm for 15 minutes. the extract was concentrated using a rotary evaporator and dried 45 in an oven.

Experimental design.

The experiment was designed to investigate the effect of aqueous extract of AG and atrazine on liver tissue and its effect on some functional blood parameters in local male rabbits. then divided into three groups, including 5 rabbits are:

1- Atrazine group 10 ml for two weeks daily.

2- Aqueous extract of AG and atrazine group from day to day at dose of 10 ml and at concentration of 30% of AG and 10 ml of atrazine and for two weeks daily.

Anatomy of animals and blood collection

The animals were then numbed with chloroform and anatomy by opening the abdominal cavity and draw blood through heart puncture to get the most amount of blood. Blood samples were placed directly into sterile anticoagulant –free test tubes its capacity 10 ml, it was left for 15-20 minutes at laboratory temperature and then transferred to a centrifuge 3000 rpm for 15 minutes for the purpose of obtaining the serum stored in the refrigerator at temperature -4 C[5(15)]for the purpose of physiological testing that including AST and ALT [16] ALP[17]. The liver was removed after fatty substances were removed fixed with formalin concentration 10% so as to preserve the cellular structure and the natural structure and the natural state of the tissue, after two days 48 hours I was extracted from formalin and washed with tap water for a (3-6) hours, then a series of operation took place to prepare tissue slides colored Eosin and Hematoxylin then the prepared tissue slides were examined using a compound microscope and under a 10X magnification force, and measured the rate of diameters of liver sinuses, the diameters of central veins and hepatocellular diameter.

Results and Discussion

Physiological study: The results of the physiological study are shown in Table 1 for atrazine group 10 ml in rabbits' serum there is a significant increase (P≤0.05) in the average level of ALT, AST and ALP enzymes for two weeks compared with the control group. The results of this study are consistent with which reached Jested and his group [18] in his study with doses rats 300mg/kg from atrazine for one month noting an increase in the levels enzymes mentioned above , consistent with the results of this study of Hussein and his group [19] which he made on rats by taking them 400gm/kg from atrazine for two weeks ,the cause of the increase in rate of enzymes levels in the blood serum indicates the extent of damage to the liver and heart tissues the result of treatment with atrazine leading to a change in hepatic metabolic functions through damage to hepatocytes or the increase may due to kidney damage due to heart damage which leads to the occurrence of chronic renal insufficiency due to necrosis, degeneration or damage to renal cells leading to enzymes leaking into the blood serum [20] Or due to increased oxidative stress resulting from the high proportion of oxidant from the liver because atrazine is an oxidizing agent capable of attacking the cell membrane and antioxidant molecules which cause damage and breakdown in DNA , protein and fat[21].consistent with this result what he pointed out Mohammad and his group [22] .to born of free radicals (ROS) Reactive Oxygen Species leads to the breakdown of mitochondria containing the enzymes listed, because oxidative stress increases the process of lipid peroxidation leading to cirrhosis.

The results of the physiological study are shown in Table 1 for atrazine group 10 ml that treatment with aqueous extract of AG at concentration in rabbits to presence of significant decrease (P≤0.05) in AST and ALT , there is no significant difference (P≥0.05) in rate of ALP for two weeks compared with the control group. Consistent with the results of our study [23]. noting when dosing mice with 10% from AG for three month leads to decrease in the concentration of enzymes mentioned above ,the results of the atrazine group with the results of this group treatment with AG to increase in average level of liver cell enzymes that lead to oxidative stress of the kidney and liver that results born of free radicals capable of attacking the cell membrane and antioxidant molecules which cause damage and breakdown in DNA , protein and fat [22]. Also the study of Ayaz and his group[24]referred to the protective role of aqueous extract go AG when give 0.5 g/kg daily for two weeks to mice treated with toxic substance Trichloroactate where they notice a decrease in the levels of hepatic enzymes mentioned above perhaps due reason to improvement in the liver functions due to increased enzymatic antioxidants such as Catalase and Glutathione peroxidase , and non- enzymatic such as vitamin A,C, and because the plant contains phenolic compounds which working on decrease free radical formation[25].

Table (1): effects of aqueous extract of AG in concentration 30% on levels rate of some enzymes in serum of rabbits that treated with atrazine.

parameters groups	AST U/L	ALT U/L	ALP U/L
Control group Normal saline (10)ml	18.80± 0.84	20.60± 1.52	23.40± 3.78
Atrazine 10 ml	27.38± 3.91	29.40± 1.52	44.60± 6.50
Atrazine 10 ml and gum Arabic 30%	16.30± 0.84	18.80± 0.84	23.46± 2.21
LSD	2.74	1.51	4.89

Histological study: Figure 1 represents the normal histological structure of the control group for two weeks for a rabbit liver, the liver consists of a central vein and hepatic cords surrounding cubic cells distributed between hepatic cord and hepatic sinuses. The results have shown the formal and histological measurements in Table 2 and Figure 2 to section of liver tissue for atrazine group treated with AG 30% to absence of significant differences (P≥0.05) in diameters rate each of hepatic sinuses, central vein and hepatic cells compared with the control group, as for the histological composition for atrazine group treated with AG 30% note a slight improvement in liver tissue represented by lack of central vein expansion compared with group treated with atrazine alone ,absence lack in sinuses and less cellular infiltration.

The study of [26] pointed to that oral dosage with alcoholic extract of AG plant 400,800mg/kg daily for one week for mice treated with toxic carbon tetrachloride prevent necrosis, fatty infiltration and liver damage resulting from this toxic substance CCL4 that the histological structure of the hepatic cords and tissue normal appears addition to low in hepatic enzymes. This confirms the effectiveness of AG as a medicinal plant used in folk medicine because it contains many effective chemical compounds such as carbohydrates rhamnose, galactose and arabinose, acids such as glucuronic and mineral salts such as calcium, potassium, magnesium and sodium, addition to medically effective compounds such as phenols, flavonoids and alkaloids [27]. Also the study of Ram and his group pointed to a role of alcoholic extract of gum Arabic plant when dosing rabbit 500mg/kg daily for forty five days leads to remain the liver, kidney, heart and aorta tissues similar to normal, addition to low in hepatic enzymes and liver lipids that pointed to the possibility of the plant in the treatment of cases of

fats and atherosclerosis[28] .

Table (2): Measurement of liver sinusoids diameters, central veins diameters and hepatocyte diameters measured in micrometers for male rabbits after dosing with aqueous extract of AG and atrazine for two weeks.

hepatocytes groups	Diameters of liver sinuses µM	Diameters of central veins µM	Diameters of hepatic cells µM
Control group Normal saline (10)ml	41.2 ± 9.1	72.7 ± 8.4	17.3 ± 2.0
Atrazine 10 ml	80.7 ± 2.3	*98.7± 209	*23.2 ± 3.1
Atrazine 10 ml and AG 30%	46.9 ± 9.1	8O.2 ± 9.0	19.9 ± 3.1
LSD	13.0	12.2	2.9

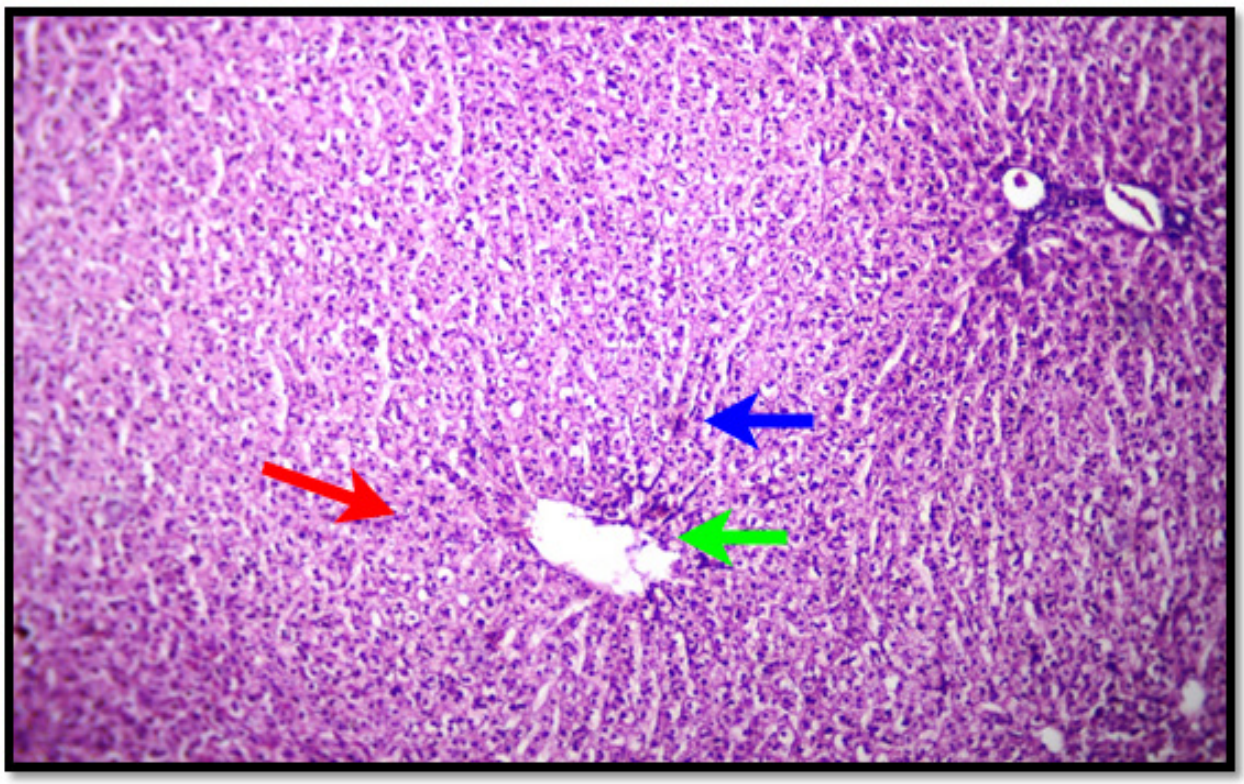


Figure 1 Section of liver tissue in control group for two weeks notes presence of the central vein (→) regularity in the hepatic cords (→) with presence of sinusoids (→) (10X H&E)

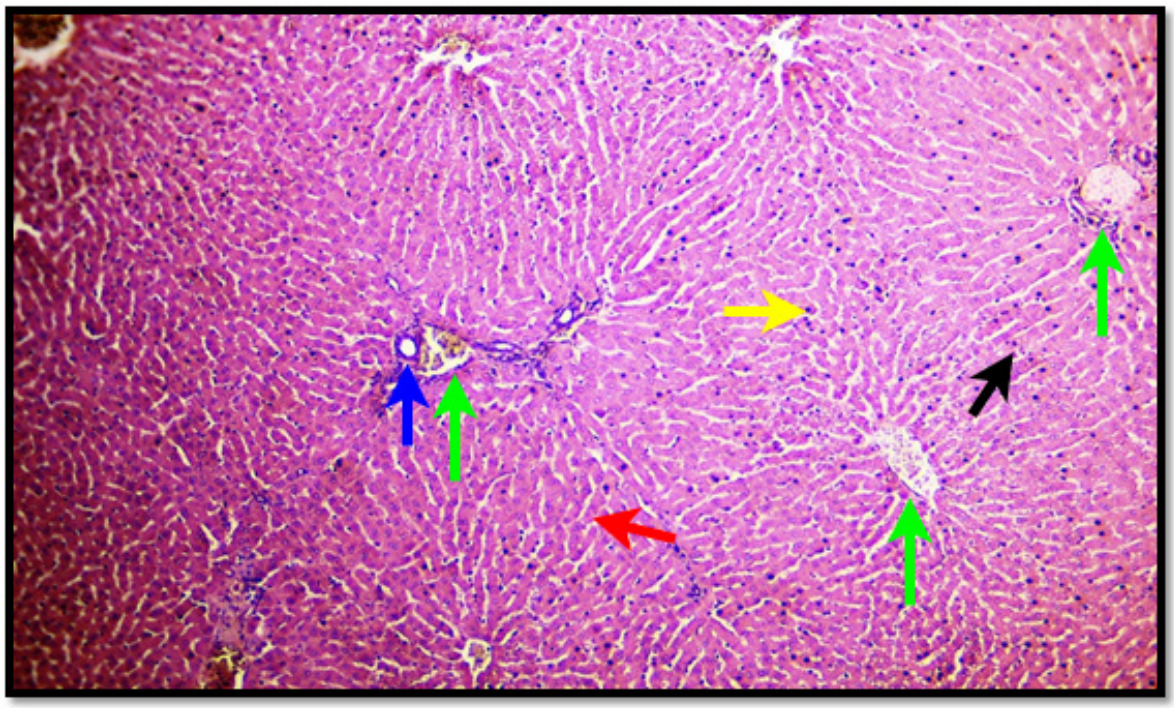


Figure 2 Section of liver tissue in atrazine group treated with 10 ml for two weeks notes presence of congestion and dilatation of central vein (→) and portal vein (→) dilatation in sinusoids (→) cellular infiltration (→) severe irregularity in hepatic cords (→) with water degeneration (→). (10X H&E)

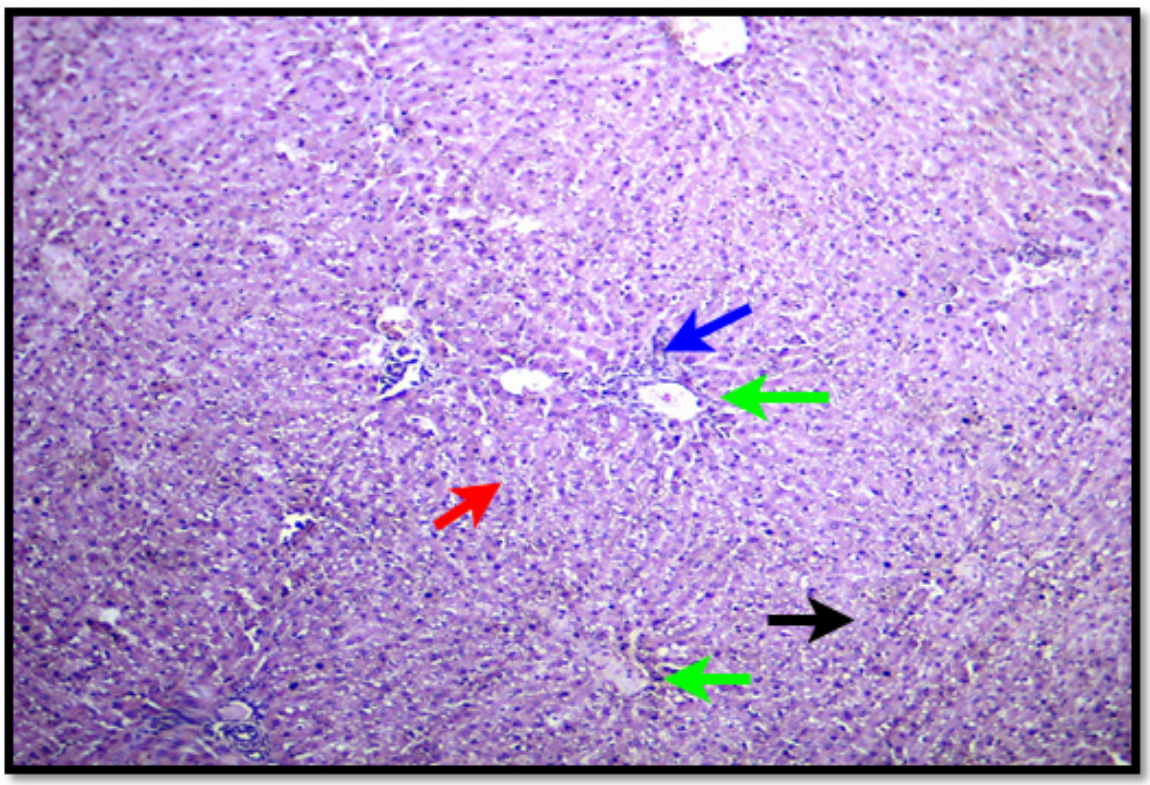


Figure 3 Section of liver tissue in atrazine group treated with 10 ml for two weeks with AG with 30% concentration notes presence of congestion and dilatation of central vein less than previous treatment (→) less cellular infiltration (→) presence water degeneration (→) dilatation in sinusoids (→). (10X H&E)

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

(1) Adebayo, A.H; Abolaji , A.O.; Opata , T.K. and Adegbrnoro , I.K. Effects of ethanolic leaf extract of chrysophyllum albidum Go. on biochemical and hematological parameters of albino wister rats. African Journal of Biotechnology. (2010). 9(14) : 2145-2150.

(2) Saeid , S. and Tariq, P. Antibacterial activities of emblica offinalis and (Corianderum sativium L.) against gram negative urinary pathogens. Pak.J. pharm Sci Jan: (2007). 20(1): 32-35.

(3) Zuhair, N.; Huda, A. and Faris, K. Iraqi Medical Herbs Guide. Ministry of Health. (1996).

(4) Cabuk, M.; Alcicek, A.; Bozkurt, M. and Imre, N. Antimicrobial properties of essential oils isolated from aromatic plants and using possibility as alternative feed additives II. National Animal Nutrition Congress.18-20 September, Konya, Turkey: (2003). 184-187.

(5) Saibara , T.; Toda , k.; Wakatsuki , A. and Ogawa. Protective effect of 3-methel - I-pyenyl-2-pyrazolin-5-one, free radical scavenger, on acute toxicity of parquet in mice. Toxicol let; (2003). 134: 51-54.

(6) Marian, A.; Maria, L. and Cloudio. Products and Applications of Biopolymer, Edited by Dr. Johan Verbeek: (2012). 1-26.

(7) Ali, B.H.; Ziada, A .and Blunden, G. Biological effects of gum Arabic: a review of some recent research. Food and Chem. Toxicol, (2009). 47: 1-8.

(8) Gluzczak,L.; Miron, D.S.; Moraes,B.S.; Simoes, R.R.; Schetinger, M.R.; Morsch, V.M.; Loro,V.R. Acute effect of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish (Rhamdia quelen). Comparative Biochemistry and Physiology. (2007). 146:519-524.

(9) Curic, S.; Gojmerac, T. and Zuric, M. Morphological changes in the organs of gilts induced with low-dose atrazine. Vet. Archiv., (1999). 69: 135-148.

(10) Hussain, R.; Mahmood, F.; Khan, M. and Muhammad, F. Pathological and genotoxic effects of atrazine in male Japanese quail (Coturnix japonica). Ecotoxicology, (2011). 20: 1-8.

(11) Lim, S.; Ahn, Y.; Song, C.; Chung, H.; Jang, C.; Par, S.; Lee, K. and Pak, K. Chronic Exposure to the Herbicide Atrazine Causes Mitochondrial Dysfunction and Insulin Resistance. PLOS ONE, (2009). 4(4):1-11.

(12) Bhatti, J.; Sidhu, I. and Bhatti, G. Ameliorative action of melatonin on oxidative damage induced by atrazine toxicity in rat erythrocytes. Mol. Cell. Biochem. (2011). 353:139-149.

(13) Harborne, J. Phytochemical methods. A guide to modern techniques of plants analysis. 2nd.ed. Chapmam & Hall, London, New York. (1984).

(15) Pinon – Lataillade, G.; Thoreux- Manaly, A.; Coffigny , H. ; Masse , R. and Soufir , J.C. Reproductive toxicity of chronic laed exposure in male and femal mice. Hum. Experiment. Toxicol., (1995). 11: 872-878

(16) Bergmeyer, H.U. and Rej , M.H. International Fedration of Clinical Chemistry (IFCC) scientific committee, Analytical section. J. Clin. Chem. Clin. Biochem., (1985). 24: 481- 495.

(17) Belfield, A., & Golderg, G. M. Revised assay for serum phenyl phosphatase activity using 4-amino-antipyrine-Enzyme., (1971). 12, 561–573.

(18) Jestadi, D.B.; phanien dra, A.; Babji, U.; Srinu,T.; Shanmuganathan, B. and Periyasamy, l. Effect of short-term exposure of atrazine on the liver and kidney of normal and diabetic rats. Journal of Toxicology., (2014). 10(11): 7.

(19) Hussain, R.; Mahmood, A.; Khan, A.; Javed, M, T.; Rehan, S. and Mehdi, T. Cellular and biochemical effects induced by atrazine on blood of male Japanese quail (Coturnix Japonica) Pesticide Biochemistry Physiology (2012). 103(1) :38-42.

(20) Fowler, P. A; Bellingham, M. and Sinclair, K.D. Impact of endocrine- disrupting compounds (EDC5) on female reproductive molecular study. Ecotoxicology and environmental safety., (2012). 78: 170-177.

(21) Xing,H.; Li, Z.; Wang, X.;Gao, S.;Xu, S. and Wang, X. Oxidative stress response and histopathological changes due to atrazine and chlorpyrifos exposure in common carp. Biochem. Physiol., (2012). *103*(1): 74-80.

(22) Mohammad, R.S.; Attabi, A.L. and Diwan, A.L. protective role of clomiphene citrate from the biochemical effects of atrazine exposure in adult male rats. *Basrah Journal of Veterinary Research.,* (2012*). 11* (2): 82-92.

(23) Babiker, Abbas.T.; Elimam, M. and Mohammad.A. Effect of Gum Arabic on liver function and antioxidant enzymes of Sprague- Dawley rats. Journal *of pharmacy and biological Sciences.,* (2017). *12* (2): 29-33.

(24) Ayaz,N.O.; Ramadan, KH.S.;Farid. H.E.A. and ALnahdi,H.S. Protective role and antioxidant activity of AG against trichloroacetate – induced toxicity in liver of male rats, *Indian Journal of Animal Research.,* (2017). *51*(2): 303-309.

(25) Verbeken, Dewettinck exudate gums: occurrence, production and application . Applied Microbiology and Bio technology ., (2003). *63*: 10-21.

(26) Singh, J.; Bias, C.S.; Hoda, M. S .and Pal, R. Hepaty protective activity of *Acacia Senegal* pod against carbon tetrachloride – induced hepato toxicity in rats. *International Journal of Pharmaceutical Science Review and Research.,* (2014). *26* (1): 165-168.

(27) Hooda, M.S.; Rishi, P.; Bhandari, A. and singh, J. Antioxidant potential and free radical scavenging activity by pod extract of *Acacia Senegal*. Int.J. Phar chem and biological sciences., (2013). 2(4):500-506.

(28) Ram, H.; Jatwa, R. and Purohit. A. Antiatherosclerosis and cardioprotective potential of *Acacia Senegal* seeds in diet – induced atherosclerosis in rabbits. Biochemistry Research International., (2014). ; 2014: 6.

Relation between Working in Petrol Station and Blood Hemoglobin Levels for the Filling Workers in Al-Najaf City/ Iraq

Fulath Abdul-Redah Muhsin

Community Health/The Head of Community Health Department / Al-Kufa Technical Institute /Al-Furat Al-Awsat Technical University

Abstract

Petrol station workers were exposed to several pollutants like air pollutants by inhalation, or skin contact with petroleum derivatives like benzene, and another port of entry. This comparative cross-sectional study was conducted to find a relationship between working in petrol stations and hemoglobin blood levels of the workers in Al- Najaf-city. The exposed worker's group was (50 male) of petrol station workers aged 20-50 years, the years of working was (1 ≥15 years) of (≥7 h/day) that included in this study, the comparison group was 50 healthy male service and office workers aged (20-50 years) matching with the study group from al-Kufa Technical Institute, Al-Furat Al-Awsat Technical University.

Blood samples were tested at the field by using a digitized portable device (hemochromax plus) which gave the blood hemoglobin concentrations at mg/dl of the petrol stations worker. The results of blood hemoglobin were compared between both groups. There was a highly statistically significant difference (HS) between Gas station workers and Control group at the *P*-value of (0.001) of Hb blood concentration levels and the mean of gas station worker and control group were (11.57 and 15.58 mg/dl) respectively. there was a highly statistically significant difference (HS) between not anemic and anemic workers at the *P*- value (0.000), and there were 47 workers (94%) out of all 50 workers had anemia after the field test.

There was a statistically significant difference (HS) between Hb levels of Gas station workers and Standard Normal Hb Level at the *P*-value = 0.007. There were a moderate inverse negative correlated between the time of exposure in the year and blood hemoglobin levels in mg/dl at the (r = - 0.57), which mean there were decreases of blood hemoglobin levels of the worker when increases of the duration of service employment. The study concluded from all results that were finding that decreased blood hemoglobin concentration than the normal value of the petrol stations workers for all stations, which might be due to the adverse effect of the workplace pollutant on bone marrow. Attention should be given by a periodical medical assessment of all workers in the petrol stations, and Obligate the petrol stations managers for supplies all personal protective equipment for all workers.

Keyword: *workplace pollutants, Hemoglobin, Anemia, Filling Workers, Al- Najaf city.*

Introduction

Health impact of occupational exposure to petrol and air pollution from the exhaust of automobile sources and un discover through petrol station workers [(1)]. Lack of ventilation &unused of the personal protective device at the work place when using benzene will arise the occurrence of Toxic effects of benzene and petrol derivatives in workers [(2)]. Repeated exposure can lead to inflammation of the respiratory tract and hemorrhage in the lung. Different air pollution like benzene and atmospheric polluted air like car exhausts, absorbed into the human body by respiratory tract or via epidermal contact [(3)].

Air pollutants and other chemicals like benzene or other heavy metals and carbon monoxide (CO) and carbon dioxide (CO_2)can Cause adverse health effects by body metabolites and interference with biochemical or Physiological processes of the human body [4]. Petroleum derivatives were used for different reasons by human beings at home, in manufacturing and petrol station [5].

Petrol station workers are exposed to a mixture of hydrocarbons in a fuel vapor through dispensing fuel and to the gases from car exhaust [6]. In the petrol station; the amount of Fuel spread as well as the ambient temperature interaction significantly to the arising the Emission of volatile hydrocarbons. Most people have a greater risk of exposure to gasoline vapors, these include petrol station workers, service station and drivers of cars[7].

The nature of the petrol station workers makes them readily available of the most time to exposed by skin or ingestion and inhalation. benzene & other derivatives affect blood production by affecting the bone marrow. the most characteristic effect resulting From intermediate and chronic benzene exposure was reduced the development of blood cell[8].

And causes aplastic anemia in human. The clinical finding in petrol. Hepatotoxicity cytopenia; which was a decrease in several cellular elements of circulating blood as Manifested as anemia; leukopenia and thrombocytopenia in humans. The inhalation of petrol derivatives like benzene vapor is rapidly absorbed into the blood and distributed Through the body. Several studies of benzene-exposed workers agreed that chronic Exposure to benzene at air resulting in the adverse hematological effects [9].

The aim of this study: To find a relationship between exposures to workplace pollutants and hemoglobin blood levels of the petrol station filling workers of Al-Najaf city.

Material and Method

Study Design: Comparative cross-sectional study.

Place of the study: The study was conducted in «Al-Najaf city that located to the South of Baghdad about 165Km, Iraq».

Period of the study: Data Collection was lasted from (30/1/2018 to 19/2/2018).

Sampling collections: All petrol stations that belong to governorate in Al-Najaf city were included In this study. The blood samples were collected from 7 government gas petrol stations in Al-Najaf city, the samples were tested at field of the petrol stations. From 50 person That working on it.

Questionnaire: A well-designed questionnaire was applied in this study.

Field tests: Samples were tested by using a portable digitized device portable, on each petrol station in Al-Najaf city. was used in this study to test the Hb % level of the petrol station workers (Hemochromax plus) of Korean made which was need only one blood drop to give the Hb levels results.

Statistical methods: Descriptive and analytical statics were carried out in This study by using a statistical package from social science (SPSS) version 18. Z-test was applied to obtain only statistical significance difference and Pearson correlation coefficient (r) was used to find the correlation between exposure time in years and hemoglobin concentration in blood.

Results

Table (1): comparison between study group of petrol station workers and Comparison group among age in years.

Feature	Study group (Filling workers) n = 50	Comparison group n = 50	P- value (z-test)
Age (years)	32.47 ± 6,7	34.54 ± 8.1	*0.47

*** (Non-Significant)**

Table (1): Show that There is non-statically Significant different between age group and comparison group at P-value = 0.47.

Table (2): Comparison between study group of petrol station workers and Comparison group of Hemoglobin concentration levels.

Parameter	Petrol station workers (n = 50)	Comparison group (n = 50)	P- value (z-test)
Hb concentration (mg/dl)	11.57 ± 1.1	15.58 ± 2.2	*0.001

*** (Statistically Highly Significant) (HS)**

Table(2): Show that there is a highly statistically significant difference (HS) between petrol station workers and Comparison group at the P- value of (0.001) of Hb blood concentration levels, and the mean of petrol station worker and the Comparison group were (11.57 and 15.58 mg/dl) respectively.

Table(3): Comparison details between normal (nonanaemic) and low concentrations (anemic) among the study group of petrol station workers of Al-Najaf city.

Nonanemic no. (%) (workers)	Anemic no. (%) (workers)	Total No. (%)	P- value (z-test)
3 (6 %)	47 (94%)	50 (100%)	* 0.000

*** (Statistically Highly Significant) (HS)**

Table(3): Show that there is a highly statistically significant difference (HS) between nonanemic and anemic workers at the P- value (0.000), and there were 47 workers (94%) out of all 50 workers had anemia after the field test.

Table(4): Summery statics comparison among a study group of petrol station workers Hb levels and standard normal Hb level in the adult.

Comparison Parameter	Test of Significance
HB levels of Gas station workers × Standard Normal HB Level	*P - value = 0.007

(Based on Z-test * S)

Table (3): Show that there is a statistical significant difference (HS) between Hb levels of petrol station workers and Standard Normal Hb level at the P - value = 0.007.

(*moderate inverse negative correlated)

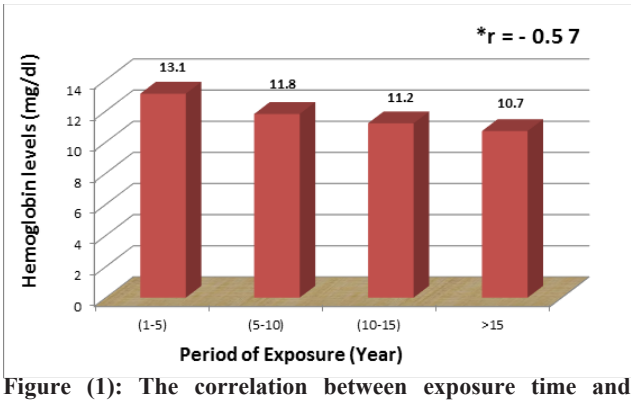


Figure (1): The correlation between exposure time and hemoglobin blood levels of the worker in the gas stations of AL-Najaf city

Figure (1): Show that there are a moderate inverse negative correlated between the time of exposure in the year and blood hemoglobin levels in mg/dl at the (**r = - 0.57**), which mean there are decreases in blood hemoglobin levels of the worker when increases of the duration of service employment.

Discussion

Air pollution and the dealing with petrol derivatives still have more priority for the worker when risk assessed, as well as its toxicological effect for the bodyworker. The study in the Al-Najaf petrol station could be considered the first study with this result finding, most of the worker of the study group were in the second decade of the life, the study group of the petrol station worker and healthy control group was matched for age to find if there is any significant difference for achieving the study aim.

All the results that we're found in the present study between the study group of the worker and the comparison group were significantly more among the petrol station workers than the other (control group) from the normal parameter.

The present study was showed a highly statically significant difference when comparison was made between the petrol station worker and control group in hemoglobin concentration levels, this defect might be due to the damage to the bone marrow by the toxic effect of the pollutant hydrocarbons gases that emissions from the car exhaust in the ambient air of the petrol stations and the entrance of the petroleum products like benzene or gasoline oil through the respiratory tract by inhalation or ingestion and entrance by skin contact way.

The results data in present study was showed that the most numbers and the percentage of the worker that represent of studied samples have anemia through the clear decrease of the blood hemoglobin concentration 47 (94%) respectively, with the higher statistical significant differences in comparison with non anemic worker, the same finding results were obtained by Okoro A.M. [10], of study in Nigeria of anemic worker in petrol stations at 2010.

While the different results were found in Gaza, Palestine with that mentioned by Sirddah M.M., et al in 2013, who found that the hemoglobin levels of the blood concentration levels were increases in the blood of the petrol stations workers [11].

The present study was showed a highly statically significant difference between Hb levels of petrol station workers and standard normal hemoglobin Level at the *P*-value = 0.007, The results agreement with that mentioned by Anthony Seaton, et al, 2016 in United Kingdom [12], who found that there was a high difference when compared with the normal Hb value. In addition, the same results were found by Tunsaringkarn T, et al, in Bangkok, at 2013, Thailand [13].

The present study showed that there was a moderate inverse negative correlated between the time of exposure and blood hemoglobin levels, (r = - 0.57), which mean there was a relationship between exposure time and blood hemoglobin levels, in another meaning the decreased in blood hemoglobin levels of the filling petrol station worker was happened when increased in years of service, this results might be due to continuity in exposure to workplace pollutants, the same finding results were obtained by Sahb AA, of study in Baghdad in 2013 [14].

Conclusions

The following conclusions can be derived from this study:

1. There was a highly statistically significant difference (HS) between gas station workers and control group at the P-value of (0.001) of Hb blood concentration levels.

2. There was (HS) between nonanemic and anemic workers at the P- value (0.000).

3. There was an (HS) between Hb levels of gas station workers and standard normal Hb Level at the P-value = 0.007.

4. There were a moderate inverse negative

correlated between the time of exposure in (years) and blood hemoglobin levels in mg/dl at the (r = - 0.57), which mean there were decreases of blood hemoglobin levels of the worker when increases of the duration of service employment.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1- kesavachandran, C.; Mathur, N.; Anand, M.; Dhawan, A.: Lung Function abnormalities among petrol pump workers of Lucknow, north India. current science. 2016; 90,1177_1178.

2- Dundar, B.; Farul Okten; Arslan, M.K.; Nanik Delibas; Bahallin Baykal; Cargalny Arslas; Mustafa Gultepe; Ishan, E.I.: The effect of long-term low_ dose Environmental Research. 2016; 101,140_145.

3- Shouren Kaung and weihui Liang : Clinical analysis of 43 cases of chronic benzene poisoning, Chemico - Biological Interactions. 2015; 153_154.

4- Qing, Lan; Luoping, Zharg; Guilar, Li; Rappaport, S.M.; Min sher; Alter, B, P.; Yongi Wo; William Kopp; Swranga Waidyanatha; Charles Rabkin; Weitong Guo: Hematotoxicity in a worker exposed to low Levels of benzene. Science. 2014; 306, 1774_1776.

5- Tsai, S.P.; Fox, E.E.; Ransdell.J.D.; Wendt, J.K.; Waddell, L.C.; Donnelly, R.P.: A hematology surveillance study of petrol chemical workers exposed to benzene. Regul. Toxical.Pharmacol. 2014.

6- Ovurd, S.S. and Ekweozor, I.K.E.: Haematological changes associated with crude oil ingestion in experimental rabbits. Afr.J.Biotech. 2014; 3:346_348.

7- Qin.W, Xu. Z, Luetal.Y. : "Mixed organic solvents induce renal injury in rats,"Plo 5 ONE. 2012; Vol.7, no.9, Article IDe 45873.

8- Hu.Y, Chen. B, Qian. J, Jin. T, Jin. L, and Lu. D.: "Occupational coke oven emissions exposure and risk of abnormal liver function: modifications of body mass index and hepatitis virus infection," Occupational and Environmental Medicine. 2017; vol. 67, no. 3, pp. 159–165.

9- S. Hussain. A Sharef D. M. S., and Mahmood N. M. A.: "Plasma proteins profile and renal function relative to the exposure time of gasoline filling station workers in Sulaimani City," International Journal of Pharmacy and Pharmaceutical Sciences. 2013; vol. 5, no. 4, pp. 334–338.

10- Okoro A.M.: Department of Physiology, College of Medical Sciences, University of Calabar, P.M.B.1115, Calabar , Cross River State, Nigeria 2011.

11- Sirddah M.M., AlLaham N.A, and. ElModhoun R.A.: "Possible health effect of liquefied petroleum gas on workers at filling and distribution stations of Gaza governorate, "Eastern Mediterranean Health Journal. 2013; Vol.19,no.3.pp.289_294.

12- Anthony Seaton, Watson JG, Chow JC, Terres IMM. : Assessing the impact of petrol stations on their immediate surroundings, United Kingdom. Journal of Environmental Management. 2016; 91(12): 2754– 62.

13- Tunsaringkarn T, Soogarun S, Palasuwan A.: Occupational exposure to benzene and changes in hematological parameters and urinary trans, transmuconic acid Bangkok, Thailand. Int J Occup Environ Med. 2013; 4: 45-49.

14- Sahb AA. : "Hematological assessment of gasoline exposure among petrol filling workers in Baghdad." Fac Med Baghdad. 2011; 53(4).

difference in combination group compared to metformin group. A significant increase in the mean of salivary BDNF in combination group as compared to metformin group was seen and there is no previous study regarding salivary BDNF in type 2 diabetic patient under therapy. In order to understand the impact of metformin on BDNF levels, [Ma et al. 2015] investigated the effect of metformin on Schwann cells under hypoxia condition and they found that the mRNA levels of BDNF were significantly decreased. However, this detrimental effect of hypoxia on gene expression in Schwann cells was partially reversed by metformin. The mRNA level of BDNF in metformin-treated Schwann cells was higher than those without metformin under hypoxia condition. This beneficial effect of metformin on gene expression under hypoxia condition was significantly inhibited by compound C, which is an inhibitor of AMP-activated protein kinase (AMPK) and an important cellular regulator of lipid and glucose metabolism[16]. Taken all together, these findings suggest that the correlation between BDNF and metformin may be the reason of metformin-induced insulin action by insulin receptor binding, metformin-induced high BDNF levels due to increasing AMPK, and enhanced tyrosine kinase receptor activity which may amplify BDNF signaling. BDNF inhibited during hyperglycemic clamp conditions in humans. This may explain the concomitant finding of low circulating levels of BDNF in individuals with type 2diabetes[17]. Significant increase in the level of BDNF in combination group as compared to metformin group may explained by good glycemic control of combination treatment as compared to metformin mono-therapy treatment[18].

Conclusion

Both monotherapy and combination therapy was affect salivary level of brain derived neurotrophic factor. Burning mouth syndrome was seen secondarily to diabetes and poor glycemic control and seen in both patients groups.

Conflict of Interest: We declare that we have no conflicts of interest.

Human and Animal Rights All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee.

Informed Consent Informed consent was obtained from all individual patients included in this study.

Source of Funding : Self-funding

References

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2014;37(Suppl. 1):S81–S90.
2. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2009; 32(Supplement 1):S62–S67.
3. LONTCHI-YIMAGOU E., SOBNGWI E., MATSHA T. E., KENGNE A. P. Diabetes mellitus and inflammation. Current Diabetes Reports. 2013; 13(3):435–444.
4. MATTSON M. P., MAUDSLEY S., MARTIN B. BDNF and 5-HT: a dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. Trends in Neurosciences. 2004; 27:589–594.
5. COHEN-CORY S, KIDANE AH, SHIRKEY NJ, MARSHAK S. Brain-derived neurotrophic factor and the development of structural neuronal connectivity. Dev Neurobiol 2010; 70: 271–288.
6. MANNI L, NIKOLOVA V, VYAGOVA D. Reduced plasma levels of NGF and BDNF in patients with acute coronary syndromes. Int J Cardiol 2005; 102:169–71.
7. SUWA M, KISHIMOTO H, NOFUJI Y. Serum brain-derived neurotrophic factor level is increased and associated with obesity in newly diagnosed female patients with type 2 diabetes mellitus. Metabolism 2006; 55:852–57.
8. NATHAN DM, BUSE JB, DAVIDSON MB, FERRANNINI E, HOLMAN RR, SHERWIN R, ZINMAN B. American Diabetes Association; European Association for Study of Diabetes. Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care. 2009; 32:193–203.
9. TOMKIN GH, HADDEN DR, WEAVER JA, MONTGOMERY DA. Vitamin B12 status of patients on long-term metformin therapy. Br Med J. 1971; 2:685-687.
10. TING RZ, SZETO CC, CHAN MH, MA KK, CHOW KM. Risk factors of vitamin B(12) deficiency in patients receiving metformin. Arch

Intern Med. 2006; 166:1975–9.

11. LAMSTER IB, LALLA E, BORGNAKKE WS, TAYLOR GW. The relationship between oral health and diabetes mellitus. J Am Dent Assoc. 2008; 139:19–24.
12. Gurvits GE, Tan A. Burning mouth syndrome. World J Gastroenterol:WJG. 2013;19:665.
13. MEDNIEKS M, SZCZEPANSKI A, CLARK B, and HAND A. protein expression in sal-ivary glands of rats with streptozotocin diabetes. *Int J Exp Pathol*. 2009; 90(4): Pp 412-22.
14. MOORE PA, GUGGENHEIMER J, ORCHARD T. Burning mouth syndrome and peripheral neuropathy in patients with type 1 diabetes mellitus. J Diabetes Complications. 2007 Nov-Dec; 21(6):397-402.
15. PIAGOU M, PIAGOS G, NIKOLAKIS G, MAZARAKIS A. Burning mouth syndrome in diabetic patient: an update on treatment methods. Diabetologie und Stoffwechsel 2007; 2 - A36.
16. MA J., LIU J., YU H., CHEN Y., WANG Q., and XIANG L. Effect of metformin on Schwann cells under hypoxia condition. International Journal of Clinical and Experimental Pathology. 2015; 8(6):6748–6755.
17. KRABBE KS, NIELSEN AR, KROGH-MADSEN R, PLOMGAARD P, RASMUSSEN P, ERIKSTRUP C, FISCHER CP, LINDEGAARD B, PETERSEN AM, TAUDORF S, SECHER NH, PILEGAARD H, BRUUNSGAARD H, PEDERSEN BK. Brain-derived neurotrophic factor (BDNF) and type 2diabetes. Diabetologia. 2007 Feb; 50(2):431-8.
18. TOSI F , MUGGEO M, BRUN E, SPIAZZI G, PEROBELLI L, ZANOLIN E, GORI M, COPPINI A, MOGHETTI P. Combination treatment with metformin and glibenclamide versus single-drug therapies in type 2 diabetes mellitus: a randomized, double-blind, comparative study. Metabolism. 2003 Jul; 52 (7):862-7.

Efficacy of Chitosan Immune Response Against *Listeria Monocytogenes* Infection in Mice

Sura Ayed Radam[1], Inam Badr Faleh[1]; Osama Faid allah Atshan[2], Mustafa Salah Hasan[3]

[1]Department of pathology, [2]Department Microbiology, College of Veterinary Medicine, University of Baghdad,
[3]Department of Internal and Preventive Medicine, College of veterinary medicine, university of Fallujah

Abstract

The present research aimed to study the effect of dietary chitosan supplementation against murine experimentally infection by *Listeria monocytogenes*.

forty mice were divided equally into 4 groups. The 1st and 2nd groups fed on diet supplement with chitosan (1mg/kg diet) and (1.5mg /kg diet) for (4) weeks respectively, While 3rd and 4th groups considered as control positive and negative groups. At (4) weeks the first three groups were inoculated intraperitoneally i/P with (0.2) ml (1×10^9) CFU/ml, while the 4th group (control negative) inoculated with (0.2) sterile normal saline.

At (7) days post infection, the result revealed diet one of mice in each control positive and treated group at (24hrs.) post infection with heavy bacterial isolation from brain, spleen and liver of infected positive group and mild to absent bacterial isolation in the 1st and 2nd group respectively.

Grossly presence of severe congestion in the internal organs with necrotic foci seen on the splenic surface of infected positive control while the characteristic feature in the treated infected group was hepatosplenomegaly.

Sever pathological changes were noticed in the infected positive control group characterized by suppurative inflammation with necrosis accompanied with lymphoid depletion and amyloid like substance deposition while the main lesion in treated infected groups showed granulomatous lesion, lymphoid hyperplasia and mononuclear cells infiltration with heavy bacterial isolation from brain, spleen and liver of infected positive group and mild to absent bacterial isolation in the first and second group respectively, We concluded that chitosan stimulated and improve the immune responses in mice against *Listeria monocytogenes* infection.

Key word: *chitosan, Listeria monocytogenes, immunized, mice, pathology.*

Introduction

Listeria monocytogenes is regular Gram-positive motile from, rod with rounded ends, its cells found as single units or short chains or may be arranged in V, L and Y forms or in palisades [(1)]. *Listeria monocytogenes* does not produce spores and capsules are not formed [(2)]. Spread in nature where, exists largely in decaying vegetation, soil, animal feces, feed and water as make it one of the major pollutants of food and play essential role in transmitted of infection between humans and animals [(3)] also infection by *Listeria monocytogenes* can be haematogenous spread directly from the mother to fetus [(4, 5)]

Chitosan is a modified natural carbohydrate polymer derived from chitin, it have many medical uses because their ability to reduce bleeding also help deliver drugs through the skin also in limiting of fat absorption[(6)], also has been bio adhesive property for that used as a safe excipient formulations of drug, it has been used in dentistry because adhere ability to hard and soft tissues also uses in orthopedics, ophthalmology and in surgical procedures, it adheres to epithelial tissues and mucus coat present on tissues surface also has a antifungal or antibacterial, antineoplastic and anticholestermic action[(7)].

Material and Method

Chitosan was obtained from university of Al-

Bahasra, collage of veterinary medicine. Commercial assorted pellets were grinded by food grinder and weighed(1) gm and (1.5)gm of Chitosan was added to each kilogram of grinded pellets mixed well and converted into paste which passed through meat grinder to mould the paste into the original pellets from, left exposed to dry in room temperature [8]. The *Listeria monocytogenes* isolate was obtained from the unit of Zoonotic diseases in the College of Veterinary Medicine, the isolate confirmed by some biochemical tests and gram stain according to [9].

A total number(n=40) male white Swiss BALB/C mice which obtain from the (National Center of Researches and Drugs Monitor in Baghdad); then divided into fourth groups. The 1st group (n=5) mice were fed on diet supplement with chitosan (1mg/kg diet) and (1.5mg /kg diet) for (4) weeks respectively, While 3rd and 4th groups considered as control positive and negative groups. At (4) weeks the first three groups were inoculated intraperitoneally i/P with (0.2) ml (1×10^9) CFU/ml, while the 4th group (control negative) inoculated with (0.2) sterile normal saline, histopathological examination of internal organs(liver, spleen and brain) were taken from both control and infected groups about (1cm3) was taken and fixed in 10% formalin saline for histopathological section which was done according to[10].

Result and Discussion

1) Gross pathological changes:

The main gross feature in control group was severe congestion in the visceral organs specially in the liver, spleen and kidney with presences necrotic foci at the edge of spleen, while treated groups show hepato-splenomegaly was the characteristic gross lesion in the treated groups.

2) Bacterial isolate and clinical signs:

No clear clinical signs noticed on experimental animals specially the treated groups were appeared healthy and well feeding. The result showed heavy bacterial isolation mainly from brain, spleen and liver of control positive groups, while mild growth to absences in other treated groups. Also the isolate was confirmed again on blood agar then we made smear from isolate and stained with grams stain.

3) Histopathological examination:

The characteristic lesion in hepatic tissue of **control positive** show aggregation of PMNCs cells in liver paranchyma (suppurative foci) mainly in portal area accompanied with atrophy of some hepatic cords together with sinusoidal dilation and cellular infiltration in their lumen, The splenic tissue showed destructive changed with variable degree of lymphoid depletion in the white pulp, other section showed formation of multiple cystic cavities containing cellular debris together with focal amyloid like substances deposition, The brain tissue expresses sever neuronal degeneration and apoptosis accompanied with nuclear pyknosis and appearance of hypertrophic swelling astrocytes (gamistocyte), another section showed irregular cystic cavities with neuronal vaculation.

While the characteristic lesion in the liver of **treated 1st group (fed on diet with 1gm\kg of chitosan)** were development of early small granulomatous lesion seen in dilated sinusoids together with proliferation of kupffer cells (figure:1), the microscopic examination in the spleen revealed mild white pulp hyperplasia with proliferation of megakaryocyte (figure:2), together with slight vacuolar changes in some neurons also the results showed moderate gliosis (figure:3).

The pathological lesion in liver of **treated 2nd group (fed on diet with 1.5gm\kg of chitosan)** characterized by focal mononuclear cells (MNCs) aggregation mainly around central vein (figure:4) while presence of follicular hyperplasia in the white pulp was the main lesion observed in splenic tissue (figure:5), while the main brain lesion in the treated infected mice characterized by focal aggregation of MNCs in brain tissue, associated with no clear lesion in the neurons seen mainly in the brain section.

The present study shown sever pathological lesion in the internal organs (liver, spleen and kidney) of the **control positive groups** these results indicate that exposed to highly virulent microorganisms overcome the innate immune system and disseminates to internal organs induce tissue damage, these observation were in consistent with [11] who explained that virulent *Listeria monocytogenes* was one of intracellular bacteria disseminated via blood stream to internal organs and induce nonspecific inflammatory reaction by production listeriolysin O which destroyed the endothelial cells of blood vessels to induce necrosis and suppurative inflammation [12]. In addition, survival and proliferation of microorganisms in the hepatic and splenic cells will

lead to the formation of infection foci that result the infiltration of alarge number of WBCs and activate neutrophil phagocytic cells to work on other resist the invading germs [13]. We also recorded depletion of white pulp of spleen of control positive group these observations may indicate that *Listeria monocytogenes* induced reduction in acquired immune response via depletion of lymphocytic cells [14], Neuronal necrosis and microcavites formation may due to excess of nitric oxide generation literal infection which is important for intracellular signaling of new transmission both inducible and constitute nitric oxide synthase (NoS) are expressed in brain cells include neural lesion, further more inflammatory cells include neutrophils, macrophages express both (NoS and iNoS) may play an important role in elimination *Listeria monocytogenes* [15]. Also the present study explain that feeding infected mice showed mild to moderate pathological lesion in the spleen, liver and brain tissue post challenge with *Listeria monocytogenes* and these lesion characterized by appearance of granulomatous lesion mainly in liver tissue this evidence was agreement with [16] Where noted that the granulomatous reaction was considered the strongest body defense against virulent microorganism's infection, furthermore there are numerous response indicate that chitosan improve the immune response [17]. Our results showed lymphoid hyperplasia in splenic tissue mainly in mice feeding with (1.5gm\kg) chitosan this indicate that chitosan elicited both humeral and cell mediated immunity and activated immune cells to secret cytokines that play essential role in initiated mature granuloma in the liver and this evidence was in agreement with [18] Who demonstrated that feeding of chitosan increase OX62+ percentage and DCs which up regulate the major histocompatibility complex class-II Ags. without expression changing of co-stimulatory (CD80 or CD86) molecules and Ag presenting cells produced TNFα and IL-12 and activation T-lymphocytes, lymphoid tissue hyperplasia in animals fed diet supplement with chitosan may due to chitosan stimulated proliferation of lymphocytic cells.

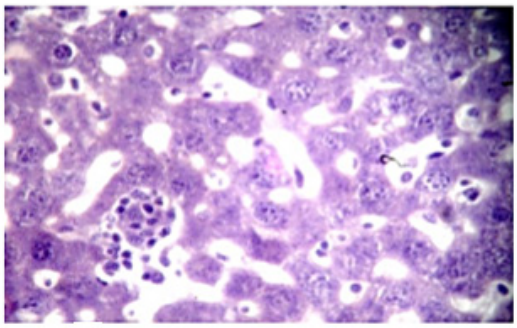


Figure 1 :histopathological section in the liver of 1st group showed small granulomatus lesion with dilated of sinusoid and proliferation of kupffer cells (H&E stainX40)

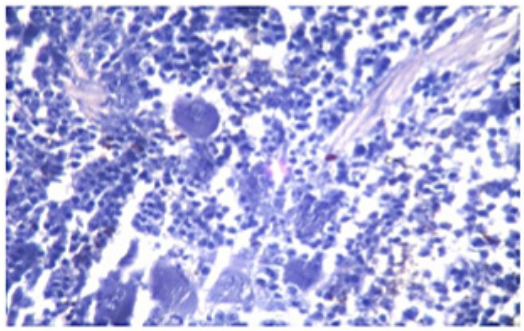
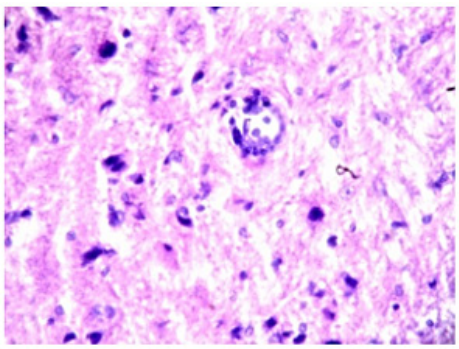
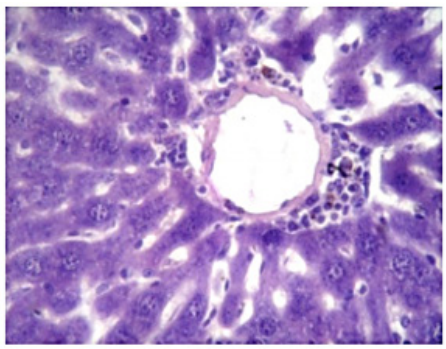


Figure 2 :histopathological section in the liver of 1st group showed moderate hyperplasia of megakaryocyte (H&E stain X40).



Figure(3):histopathological section in the liver of 1st group showed focal MNCs aggregation with slight gliosis (H&E stainX10).



Figure(4):histopathological section in the liver Of 2nd group showed MNCs mainaly around c.v wit kupffer cell proliferation (H&E stainX40)

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq**.**

Conflict of Interest: The authors declare that they have no conflict of interest.
Funding: Self-funding

References

1. Elliot T, Ryser Elmer H and Marth. *Listeria*, Listeriosis and food Safety.3 Ed. Taylor & Francis Group. 2007; LLC, CRC Press.
2. Seelige R and Bockemühl J. Kritische Untersuchungen zur Frage einer Kapselbildung bei *Listeria monocytogenes*. Zbl. Bakteriol. Parasit. Infekt. Hyg., I. Orig. 1968; 206:216–227.
3. Allerberger F and Wagner M. Listeriosis: a resurgent foodborne infection. Clin. Microbiol. Infect. 2010; 16:16-23.
4. McLauchlin J and Low J C. Primary cutaneous listeriosis in adults: an occupational disease of veterinarians and farmers. Vet. Rec. 1994; 135:615-617.
5. McLauchlin, J.; Mitchell,T .;merdon, J. and Jewell, K. *Listeria monocytogenes* and listeriosis: a review of hazard characterisation for use in microbiological risk assessment of foods. Int. J. Food Microbiol. 2004; 92:15-33.
6. Hadwiger LA. Multiple effects of chitosan on plant systems: Solid science or hype. *Plant Scienc.* 2013; 208: 42–49.
7. Dutta, P.K.; Dutta, J. and Tripathi, V.S. Chitin and Chitosan: Chemistry, properties and Application. J. Scientific and Industrial Res., 63: 20-31.dv. Immunol.2004; 84*:*131–179.
8. Salwa A S. Effect of hypercholesterolemia , chitosan and whole sonicated *E.coli* Ags on immune response and pathological changes in mice infected with *E.coli* (O:127) isolated from children suffering from diarrhea. Thesis faculty of veterinary medicine ,Baghdad university.2012.
9. Quinn P J , Markey B and Carter GR.Clinical Veterinary Microbiology. 2004; 2nd ed.,MosbyInt.,USA.
10. Luna GL. Manual of Histologic staining methods of the armed forces Institute of Pathology. McGraw-Hill Book Company, 1968; New York, USA. 3rd edition.
11. Dunn P L and North R J. Early gamma interferon production by natural killer cells is important in defense against murine listeriosis. Infect. Immun. 1991; 59:2892–2900.
12. Vazquez-Boland J, Kuhn M, Berche, P, Chakroborty T, Domanguez- Bernal G, Gonzalez-Zorn B, Wehland J and Kreft J. Listeria pathogenesis and molecular virulence determinant. Clin. Microbiol, Rev. 2001; 14: 584-640.
13. Portnoy D A, Auerbuc U and Glomski J. The cell biology of *Listeria monocytogene* infection:the intersection of bacterial pathogensis and cell mediated immunity. J. Cell. biol. 2002; 3: 409-414.
14. Zenewicz LA and Shen H. Innate and adaptive immune responses to *Listeria monocytogenes*: a short overview .J Micro and Infect, 2007; 9:1208-1215.
15. Shin T, Weinstock D, Castro MD, Acland H, Walter M, Kim H, Ahn M and Purchase H G. Neuronal constitutive and inducible Nitric oxide synthase expression in the brain of *Listeria monocytogenes*-infected cattl. ACTA VET. BRNO, 2001; 70: 43–47.
16. Enurah L U, Aboaba O O, Nwachukwu SCU and Nwosuh CI. Histopathological changes in the liver of mice challenged with *Listeria monocytogenes* in six zones of Nigeria. Journal of Experimental Biology and Agricultural Sciences, 2013; 1:1-15.
17. Porporatto C, Ismael D, Bianco A, Cabanillas M and Correa SG. Early events associated to the oral co-administration of type II collagen and chitosan: induction of anti-inflammatory cytokines Int. Immunol. 2004;16 : 433-441.
18. Carrian P, Bianco ID and Correa SG. Local and systemic activity of the polysaccharide chitosan at lymphoid tissues after oral administration. J Leukoc Biol. 2005; 78:62-69.

Fatal Drowning in Delta State, Nigeria: A Retrospective Study of Cases in this Region

Uchendu O J,[1] Ijomone E A,[2] Nwachokor F N[3]

[1]Senior lecturer, Department of Morbid Anatomy, Delta State University, Abarak, [2]Hospital Consultant, Department of Pathology, Central Hospital, Warri, Delta State, [3]Senior lecturer, Department of Morbid Anatomy, Iginedion University, Okaba, Edo State

Abstract

Introduction: Drowning death is a preventable, under-reported public health problem resulting from respiratory insufficiency secondary to immersion or submersion in liquid.

Aim: To study the sex, age, and place of death of victims of fatal drowning in Warri, Delta state, Nigeria.

Material and Method: This is a descriptive retrospective study of cases of fatal drowning reported to the coroner and subjected to postmortem examination by the authors in Delta State from 1st January 2003 to 31th December 2016. Basic information such as the age, sex and place of death were extracted and analyzed using Microsoft Office Excel, version 2007.

Results: Thirty-seven victims comprising of 34 males and 3 females were examined during this study, giving a mean incidence of about 2.5 cases per annum. Their ages ranged from 1.5 to 59 years with a mean of 28. 53 years and a dual peak in the 3rdand 4th decades. All the deaths were of accidental causes, with most the of them (70%) occurring within natural water bodies.

Conclusion: The study showed that drowning death is relatively common and usually of accidental etiology. Young males in their thirties and forties are the most vulnerable victims, with the natural water bodies being the most common site of drowning. Being a preventable cause of death, adopting and enforcing preventive safety measures by the individuals, community and government will invariably reverse this trend.

Key word: *Drowning, Medicolegal, Preventable death*

Introduction

According to statistics from WHO, unintentional injuries account for 3.9 million deaths annually with about 90% of these cases occurring in low and middle income countries (LMIC). These are attributed mostly to road traffic accident (RTA), fall, drowning, poisoning and burn.[1]

Drowning is currently defined as "the process of experiencing respiratory impairment from partial or complete submersion/immersion in liquid". Its outcome may be fatal (death) or non-fatal, the latter of which may be with or without morbidity.[2]

Corresponding author:
Uchendu Jude Obiora
e-mail: nwachokor2015@gmail.com

Though preventable, fatal drowning has become a serious public health issue globally ranking among the three leading causes of injury related death in most countries.[3] According to WHO report, drowning accounted for about 372,000 deaths in 2012, with 91% of these deaths occurring in LMIC.[4] Children have been shown to be particularly susceptible with about 450 dying daily from drowning, and a significant number suffering from varying grades of morbidities. [5] Sadly, the African continent accounts for the highest drowning mortality with a rate 13.1 per 100,000 population.[6]

Delta State of Nigeria is unique because of numerous unprotected natural water bodies with 35% of its 16,842 square kilometers total land area being riverine. It also has a high density of streams, ponds, lakes, creeks as well as the large body of ocean waters.[7]

Despite the burden of drowning death, there is a paucity of research on this subject matter in this region. This study is aimed at analyzing the age, sex and death-place of victims of fatal drowning, examined by the authors during the study period. Being the earliest of such study in this part of the world, we hope the findings will increase public and government awareness of magnitude of the problem, guide government policy formulation, contribute to literature and lastly suggest direction for subsequent research.

Materials and Method

This is a 14-year descriptive retrospective study of drowning deaths recorded by the authors in Warri. Delta State from January 2003 to December 2016.

All medicolegal autopsies performed in this region by the authors were reviewed and confirmed cases of drowning identified for this study. The information used for this study includes the age, sex, circumstance and site of death of the victims. This information was subsequently analyzed using excel spread sheet and presented in tables.

Exclusion criteria: All cases of post-mortem drowning as well as cases of doubtful history were excluded from the study.

Result

In this retrospective study, 1121 medicolegal autopsies were analyzed, out of which 37(3.3%) of the cases were as a result of drowning.

The age distribution of victims is shown in table 1, ranging from 1.5 years to 59 years, with a mean age of 28.53 years and two unimodal peaks occurring in the 3rd and 4th decade.

The sex distribution of the victims is shown in table II, with males and females accounting for 34 (91.9%) and 3 (8.1%) of the cases respectively.

Table III showed the places where the drowning occurred. Natural waters (Oceans, creeks, rivers, streams and lakes) were the site of occurrence in 26 cases (70.3%). Interestingly, two of these deaths took place during immersion baptism. Three cases (8.1%) occurred in water well. Coincidentally, two cases each (5.4%) were recorded in four different setting during the study namely: fish pond, pipeline excavation pits, gas tanks and swimming pools.

Table 1 Age distribution of victims of death by drowning

Age of victims (years)	Frequency	Percentage (%)
0-10	2	5.4
11-20	6	16.2
21-30	12	32.4
21-40	12	32.4
41-50	3	8.1
51-60	2	5.4
Total	37	100

Table II Gender distribution of the Victims

Gender of victims	Frequency	Percentage (%)
Males	34	91.9
Females	3	8.1
Total	37	100

Table III: Place of Occurrence of fatal drowning

Place of Occurrence	Frequency	Percentage (%)
Fish pond	2	5.4
Gas tank	2	5.4
Excavated Pit	2	5.4
Natural bodies of water (Rivers/Lakes/Creeks)	26	70.3
Swimming pool	3	8.1
Wells	2	5.4
Total	37	100

Discussion

Thirty seven (3.3%) of the medicolegal deaths in the study population were drowning death. This figure is intermediate between 2.2% reported by Nwafor and Akhiwu [8] in Benin City, Nigeria and 4.3% reported by Ngbea et al[9] in Makurdi, Nigeria.

We are of the opinion that this is a gross underestimation of its burden because of endemic inefficiency at data collection often seen in developing countries, and the poor attitude towards reporting accidental deaths to the police. The trend of practice where relatives of the deceased are made to pay for the autopsy has also not encouraged these relatives to report such cases to the police.

This study showed that males were found to be 11.3 times at higher risk, than the females. The high male to female ratio observed in this study concurs with reports from 62 articles in English literature reviewed on this subject matter in which a mean male-female ration (MFR) of 3:1 was observed. [10] This male predominance may be attributed to their greater involvement in boat-driving and other boat-centric jobs, including fishing and trading. Inherently, the males are more aggressive, competitive, and take on to riskier activities and behaviors some of which include alcohol, drug abuses and swimming alone, all of which may further increase the risk of drowning. [8,11,12,13] Activities in the water bodies such as crude oil theft and sea piracy which are common activities in this region may in no doubt contribute to this ugly trend. [14]

A double unimodal peak was observed in the 3rd and 4thdecade, with each peak representing 32.5% of the cases. This is most likely as a result of the active lifestyle of this age group. Our observation compares with those from Benin City, Nigeria, where the highest number of victims were in their 3rd decade,[8] but contrasts with report from an earlier study in the Niger Delta region which recorded the highest number of cases in the 6th decade of life.[12] Drowning death risk is highest among children globally.[6] In the index study, 18.9%% of the victims were children, majority of which were in their 2nd decade of life. This is lower than earlier report among children in Benin City and Niger Delta region. Lapses in supervision especially from parents has always been the major explanation to drowning among children.[8] The lower incidence of childhood drowning death in Nigerian series, relative to the global trend, may be attributed to case underreporting as natives in the study population attach less significance to death of children than that of adults.

Environmental factors, culture, behavior of the people as well as the geography of the environment play remarkable roles in the setting of drowning death. Delta state has a rich network of natural body waters, with a lot of transportation, recreational, fishing and trading activities.[8,11] This may explain the occurrence of most cases of drowning death in these natural water bodies. Our report is similar to findings in Bangladesh with 95% of cases reported in a similar setting.[14] Likewise, Sheikhazadi in Iran,[13] and Pal et al[15] in India reported 83% and 81.8% cases respectively in fresh water setting.

Our study also concurs with an earlier report by Seleye-Fubara in Niger Delta region.[12] On the contrary, Nwafor et al reported lower frequency of drowning death in natural water bodies in Benin, a city that has fewer rivers within its geographic boundaries.[11]

The two cases of drowning during baptism recorded in this study is a cautionary call to the church as it underscores the imminent danger of such activities. There is need to re-orient the church to avoid such practices where possible and if unavoidable, to adopt preventive measures including use of protective devices and employing divers to avert grave consequences. Arresting and prosecuting church leaders will act as a deterrent to others.

Public swimming pools were the second most common site (8.1%) of drowning death in this study. This is a reflection of proliferation of swimming pools in modern hotels and bars, probably an influence of westernization of our culture. We think this is mainly as a result of misadventure and recklessness of the victims, who are predominantly young. It is a general observation that these setting encourage alcohol and drug use, the role of these substances in the risk of drowning may not be overemphasized. Earlier reports have shown that swimming under the influence of alcohol is associated with increased risk of drowning death.[13] In USA, the highest rate of drowning occurred in swimming pools, which disagrees with our report.[15] In Japan, the highest drowning death occurred in bathtubs, mostly affecting the elderly persons.[16] Enforcement of fencing of Public swimming pools and use of personal floating devices routinely are worthwhile preventive measures.

Wells, fish ponds, pits and gas tanks accounted for the remaining settings for drowning death in this study. These settings are all products of man's activities and interference with the natural environment. Therefore, using barrier fencing, caution signs, early filling up of ditches and pits, especially at sites of road construction will no doubt prevent such accidents.

With respect to forensic etiology, accidental drowning accounted for all the cases. Sheikhazadi in Iran,[13] Nwafor in Benin, Nigeria,[8] and Seleye-Fubara in Niger Delta region, Nigeria[12] observed that most cases were of accidental causes. The rarity of homicidal drowning may not be unconnected with the difficulty in drowning a healthy conscious adult. As in our study, suicidal drowning was not reported in other Nigerian studies. [8,12,13] Though there has not been any study on suicide in this region, earlier studies among Nigerian Yorubas (a major ethnic group in Southern Nigeria) showed a strong negative attitude towards Suicide.[17.]

In conclusion, the study showed that drowning death is relatively uncommon and all of accidental etiology in this study. Young males in their thirties and forties are the most vulnerable victims, with the natural water bodies being the most common site of drowning. Being a preventable cause of death, adopting and enforcing preventive safety measures by the individuals, community and government will invariably reverse this trend.

Limitation of the study:

The relatively small sample size, under-reporting of the cases, the effect of co-morbid conditions, drugs and alcohol may interfere with the outcome of this report.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: Ethical approval was obtained from the ethical clearance committee of Delta State Hospital management Board/Central Hospital, Warri (reference CHW/ECC VOL1/124).

References

1. World Health Organization. The Global Burden of Disease: 2004 Update. Geneva: World Health Organization; 2008. Available at http://www.who.int/healthinfo/global_burden_disease/GBD_report_2004update_full.pdf?ua=1
2. Van Beeck E F, Branche C M, Szpilman D, Modell J H, Bierens J J L M. A new definition of drowning: towards documentation and prevention of a global public health problem. Bulletin of the World Health Organization 2005; 83:853.
3. WHO, World Health Organization. Drowning Fact Sheet No 347. Geneva: World Health Organization; 2014.
4. World Health Organization. Global report on drowning: preventing a leading killer. World Health Organization; 2014
5. UNICEF. Convention on the rights of the child. 1989; http://www.unicef- irc.org/portfolios/crc.html. Accessed October 24, 2016.].

6. Pedan M, Oyegbite K, Ozanne-Smith J,Hyder A A, Branche C, Fazler Rahman A K M,et al.World report on Child injury prevention. Geneva, Switzerland: World Health Organization; 2008.
7. Overview of Delta State-Niger Delta Budget Monitoring Group. Available at www. nigerdeltabudget.org/new/index.php/overview-of-delta-state.
8. Nwafor C C, Akhiwu W O. The Pattern and Frequency of Drowning Autopsies in Benin City, Nigeria. Annals of Biomedical Science. 2014; 13(2): 20-25.
9. Ngbae JA, Vhritherhire RA, Ojo BA, Akpor IO, Nyago T, Ugbajo BA, et al. Medico-legal autopsies in Benue State University Teaching Hospital, Makurdi North Central Nigeria: A 5 year review.2018;5(2):641-645.
10. Tyler M D, Richards D B, Reskr-Nielsen C, Saphafi O, Morse E A, Carey R. The epidemiology of drowning in low- and middle-income countries: a systematic review. BMC Public Health. 2017; 17:413
11. Kobusingye O, Guwatudde D, Lett R. Injury patterns in rural and urban Uganda. InjPrev 2001; 7(1):46.
12. Seleye-Fubara D. Nicholas EE, Esse I. Drowning in the Niger Delta region of Nigeria. Niger Postgrad Med J. 2012;19(2): 111-114.
13. Sheikhazadi A, Ghadyani G H. Epidemiology of drowning in Isfshan province, center of Iran.J Res Med Sci. 2009:14(2); 79-87.
14. Odalonu Happy Boris. The Upsurge of Oil Theft and Illegal Bunkering in the Niger Delta Region of Nigeria: Is There a Way Out? ; Mediterranean Journal of Social Sciences.2015;6(3):563-571.
15. Saluja G, Benner RA, Trumble AC, et al. Swimming pool drownings among US residents aged 5–24 years: understanding racial/ethic disparities. Am J Public Health. 2006; 96:728–733.
16. Yoshioka N, Nibe H, Enzan K, Shigeomi S. Sudden death in the bathroom and its medico-legal issues. Res Pract Forensic Med 1998;41:353–9. (in Japanese).
17. Lester D, Akande A. Attitudes about suicide among the Yoruba of Nigeria. J. Soc. Psychol.1994; 134:851-834.

Measurement of Effective Dose Detox for Workers Exposed to Benzene Toxins in Sidoarjo Paint Industry

Vivi Budiarti[1], Charles Kimura[1], Ayu Prima Kartika[1], Achmat Kuncoro[1], Abdul Rohim Tualeka[1]

[1]Department Of Occupational Health And Safety, Public Health Faculty,Airlangga University, Surabaya, Indonesia

Abstract

One of the aromatic hydrocarbon compounds, namely benzene. Benzene is a colorless liquid with a sweet odor (sweet odor), volatile in the air, soluble in water and flammable. Today the use of benzene used by the paint manufacturing industry. However, the concentration of benzene that exceed standards required threshold value will negatively impact the health of workers in Sidoarjo Paint Industry. Benzene can threaten the safety and human health if inhaled because it can damage the blood forming system profile in humans. The purpose of this research is to reduce the exposure of benzene by determining the effective dose of toxin detox benzene in the paint industry, Sidoarjo, East Java. This study was an observational study with cross-sectional approach.result the average concentration of benzene in the workplace respondents was 3.28 mg / m^3. The average effective dose should be consumed by the respondent for the avocado intake is 0,011 mg / day, chicken intake 0,026 mg / day, intake of grapefruit 0.033 mg/day, and carrot intake was 0.063 mg /day.

Keywords: *Benzene, an effective dose of detox, paint factory worker*

Introduction

One of the aromatic hydrocarbon compounds, namely benzene which has many uses for human life, especially in the industrial sector[14]. Epidemiological studies prove benzene as a carcinogen substance, have toxic effects on the blood and bone marrow. The International Agency for Research on Cancer (IARC) classifies benzene into the group-1A carcinogen material, a material that proved to be carcinogenic to humans. Contained toxic levels of benzene contained in the human circulatory system can cause leukemia[2]. NIOSH (National Institute of Occupational Safety and Health) said that workers exposed to organic solvents, it is estimated to reach 9.8 million people, mainly workers who work on the production of paints, adhesives, glues, coatings, grease solvent / cleaning materials, production dyes, polymers, plastics, textiles, printing inks, agricultural products, and pharmaceuticals [1,2]. NIOSH estimates that more than 2 million workers in the United States the possibility of exposure to benzene.[1]. In Indonesia, definitive data regarding the effects of benzene on workers association has not been found [14].

Benzene includes components of gasoline and toxins commonly regarded as the work [16]. Benzene is a toxic chemical that can lead to acute myeloid leukemia [10,12]. Benzene when oxidized in Phase 1 will produce benzene quinone, which is a source of increased toxicity. To complete benzene required in Phase 2 detoxification enzymes CYP2E1 [11]. metabolic enzymes play an important role in the activation or detoxification benzene [15]. Benzene is initially oxidised to benzene oxide by the liver cytochrome P450 2E1 (CYP2E1) in the liver[14].

BO detoxification in vivo occurs by reaction with glutathione, some metabolites of benzene aromatic rings opened or catalyzed by enzymes II metabolites such as glutathione-S-transferase (GSTs) or to form the derivative is less toxic or non-toxic and excreted through the urine [17]. To reduce or even eliminate toxins in the chemical, diperlukan4 biotransformation process. High levels of antioxidants needed to neutralize these substances, compensate for the damage caused by free radicals and protects the liver and cells of the CYP2E1 [11]. Foods rich in beef liver, brain, and salmon; Sulfation

Corresponding Author:
Abdul Rohim Tualeka,
Department of Occupational Health and Safety,
Faculty of Public Health, Airlangga University, 60115
Surabaya, East Java, Indonesia. E-mail: abdul-r-t@fkm.unair; Handphone: +6281333519732

rich foods are eggs, sulfation of chicken, beef and tuna, and foods rich in glutathione are avocado, asparagus, carrots, tomatoes, oranges, and broccoli [9].

Research using food to detoxify benzene is still very limited. Therefore, this study aimed to determine the CYP2E1 enzyme-rich food intake, sulfation, and glutathione required to detoxify benzene in every worker in Sidoarjo Paint Industry.

Benzene is one of the aromatic hydrocarbon compounds are widely used in the rubber industry, oil refining, chemical plants, shoe factories, and oil-related industries. Benzene is also found in public facilities such as cigarette smoke, gas stations, fuel burning cars, and so on. According to the Agency for Toxic Substances and Disease Registry (ATSDR), hazardous and toxic chemicals contained in oil content, namely benzene, toluene, xylene, ethylene, TPH (Total Petroleum Hydrocarbon) and Polycyclic Aromatic Hydrocarbon (PAHs). Of the six chemicals benzene exposure are very serious impact on health.

American Conference of Government Industrial Hygienists (ACGIH) in 2006 set the Threshold Limit Value (NAV) of the chemical benzene in the workplace that is the maximum value allowed is 0.5 ppm and certainly belonged carcinogenic to humans (A1 = Confirmed Human carcinogen) 14. National Institute for Occupational Health and Safety (NIOSH) set the recommended exposure limit or REL (recommended exposure Limit) for 8 hours of work that is equal to 0.1 ppm. In Indonesia alone, according Permenakertrans 13 / MEN / X / 2011, 2011, NAB allowable benzene is 0.5 ppm.6 While based on the Minimum Risk Level (MRL) set for the ATSDR 2007 was 0,009 ppm benzene every day to give effect acute and 0,003 ppm every day cause chronic effects [2].

Workers in the paint industry is working populations that have high levels of benzene higher risk of exposure, especially through inhalation in a continuous exposure period. Workers in the paint industry is constantly exposed to benzene because it is in an environment which emit benzene coming from the engine fuel pump when filling the fuel oil, fuel oil storage facilities as well as issued by the exhaust of vehicles during refueling queue. ATSDR (2007) states that the main exposure occurs via inhalation, although the dermal exposures (contact with the skin) and oral sex also may occur. Effects of acute exposure to high levels of benzene can occur immediately after exposure. Then the high concentration of benzene exposure (at least 200 ppm) are repeated can cause permanent damage to the central nervous system. Chronic exposure to benzene in the workplace associated with hematological disorders (such as thrombocytopenia, aplastic anemia, pancytopenia, and acute leukemia) (ATSDR, 2007).

Areas of the paint industry as a working environment that has a high exposure to benzene, supposedly necessary efforts to reduce the negative effects of exposure to benzene so as not to cause health problems for workers. Based on previous studies of benzene in the workplace, has not done research on effective dose detok toxic benzene in the workplace that have exposure to benzene. Therefore, the authors are interested in discussing the size of an effective dose of toxic benzene in the area detok paint industry.

Material and Method

The subjects are workers in the paint industry Sidoarjo. Criteria for inclusion in this study were male workers who had worked in the paint industry and are willing to be used as respondents. The sample was 24 people.

The variables that need to be calculated first is the effective dose intake needed by each individual to calculate the weight, length of employment (years), worked on average each day (hour), and the working time in a week (days) of the respondents, as well as the measurement of the concentration , benzene at five points in the industry. weight measurement using the scale body weight. Working length measurement, the average work every day, and time workweek obtained through in-depth interviews with respondents. Then, the measurement of the concentration of benzene in the workplace by using the NIOSH 1501 method of measurement with activated charcoal carbon pipe using the technique of gas chromatography (NIOSH 1501, 2003). The study was approved by the Ethics Committee of the Faculty of Public Health.

Then, look for the intake (formula) with the following formula:

$$intake\ non\ carsinogen = \frac{C \times R \times tE \times fE \times Dt}{Wb \times 30 \times 365}$$

C = concentration of benzene (mg / ml)

R = benzene reaction rate (m3 / h)

te = working time / day (hours)

Fe = working time / week (day)

Dt = working time (years)

Wb = weight (kg)

Calculate the effective dose per day with the calculation results noncarcinogen intake (intake) above, using the formula below:

$$effective\ dose\ of\ food = \frac{intake}{days}$$

$$= \frac{intake\ toxin \frac{\square}{\square}\ day\ x\ Mr\ enzim\ detox}{Mr\ toxin}$$

Findings

Benzene concentration (n = 24)

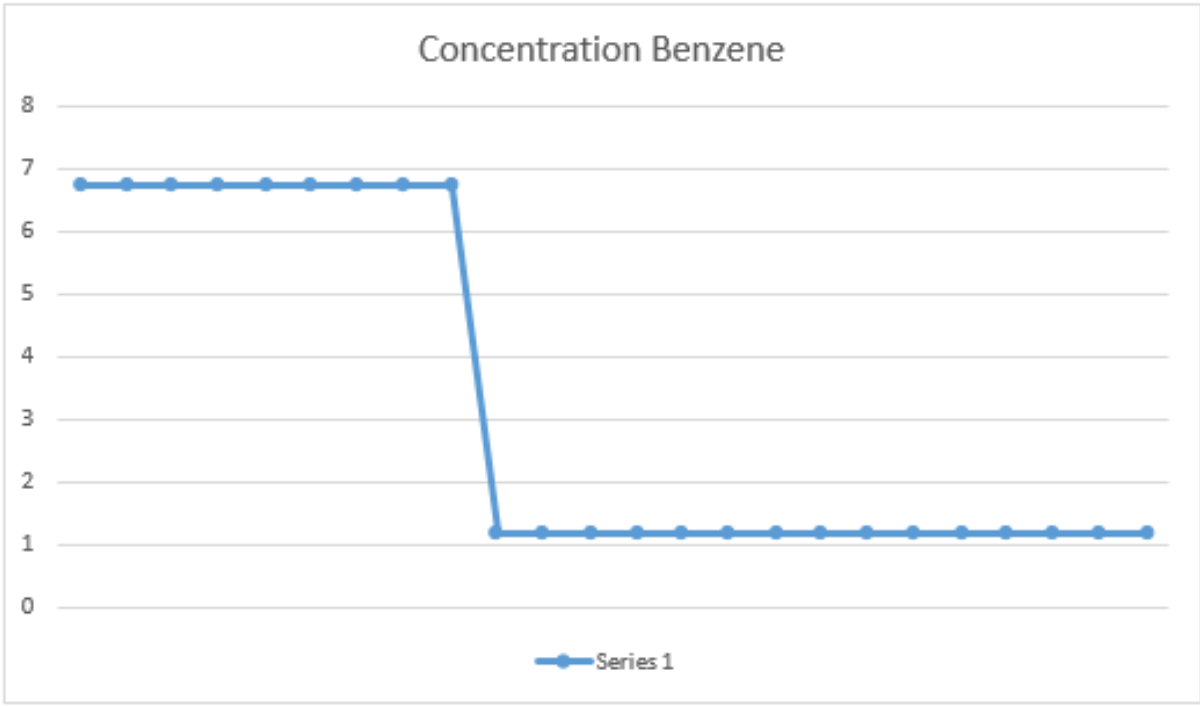


Figure 1. Concentration Benzene

In Figure 1, shows that the benzene concentration maximum of 6.7508 mg / m3 and the lowest of 1.1975 mg / m3. So that the average benzene in Sidoarjo paint industry was 3.28 mg / m3.

Comparison of Predicted Benzene intake Detox

Table 1. Comparison of Predicted Benzene Detox intake (Intake of Beef Liver, Brain Cow, Fish Salmon) (n = 24)

C (Mg / m3)	DE Avocado	DE Chicken	DE Orange	DE Carrot
6.7508	0.028338827	0.067493999	0.083412395	0.157887748
6.7508	0.03438338	0.081890187	0.101203911	0.191564545
6.7508	0.034359332	0.081832912	0.101133127	0.191430562
6.7508	0.035142143	0.083697317	0.103437251	0.195791938
6.7508	0.042399454	0.100981906	0.124798393	0.23622553
6.7508	0.024928185	0.059370943	0.073373524	0.1388856
6.7508	0.035142143	0.083697317	0.103437251	0.195791938
6.7508	0.029900104	0.071212462	0.088007854	0.166586294
6.7508	0.031630783	0.075334385	0.093101928	0.17622865
1.1975	0.001219578	0.002904644	0.003589702	0.006794793
1.1975	0.00168381	0.004010295	0.004956119	0.009381225
1.1975	0.001387943	0.003305635	0.004085266	0.007732825
1.1975	0.002968493	0.007069999	0.008737452	0.016538748
1.1975	0.000866271	0.00206318	0.002549779	0.004826368
1.1975	0.00182267	0.004341015	0.005364839	0.010154874
1.1975	0.00219233	0.005221428	0.006452896	0.012214411
1.1975	0.002703563	0.00643902	0.007957657	0.015062708
1.1975	0.001375232	0.003275361	0.004047852	0.007662005
1.1975	0.000432937	0.001031118	0.001274306	0.002412079
1.1975	0.002798375	0.006664833	0.008236727	0.015590948
1.1975	0.000396461	0.000944244	0.001166943	0.002208856
1.1975	0.000186725	0.000444719	0.000549605	0.001040324
1.1975	0.002798375	0.006664833	0.008236727	0.015590948
1.1975	0.00182267	0.004341015	0.005364839	0.010154874
3.28	0.011315372	0.026949587	0.033305622	0, 063 042 784

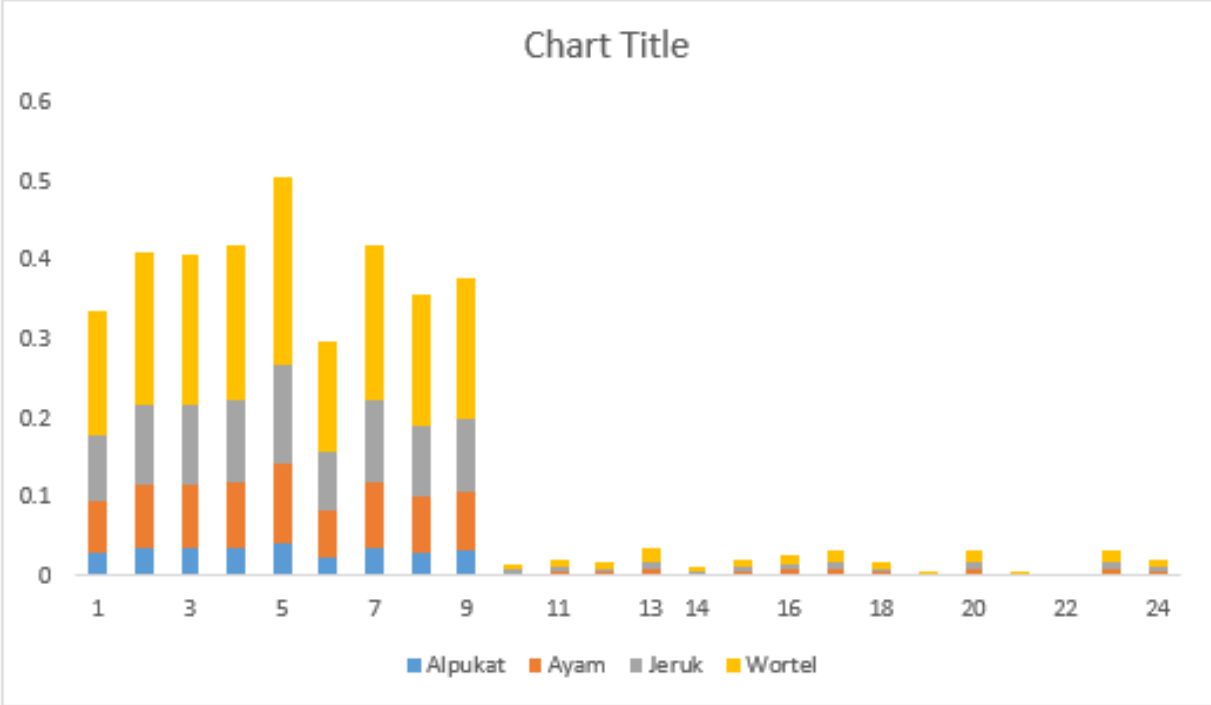


Figure 2. Comparison Prediction Detox benzene intake (Intake Avocado, Chicken, Orange, Carrots) (n-24)

In Table 1 and Figure 2, shows that the highest benzene concentration of 6.7508 mg / m3 and the lowest of 1.1975 mg / m3. So that the average benzene in Sidoarjo paint industry was 3.28 mg / m3. The average effective dose should be consumed by the respondents to the intake of avocado is0,011 mg / hr, chicken intake of 0,026 mg / day, The intake of citrus 0,033 mg / hr, and carrot intake was 0.063 mg / hr.

Discussion

Detoxification is the process that is toxic to less toxic compounds. The detoxification process to be non-toxic eliminated through urine and bile. Food-based nutrition continues to be investigated for its role in modulating the metabolic pathways involved in the detoxification process. Some of the publications that have used the cell, animal and clinical studies indicate that the component-based foods and nutrients can modulate the process of conversion and excretion of toxins from the body.

YP2E1, Sulfation, and glutathione are enzymes that work on phase 2 of the biotransformation [9]. CYP2E1 are involved in detoxification reactions-activation of a variety of endogenous and exogenous compounds. Rat hearts showed that the effect of the amount of protein in CYP2E1 activity increased CYP2E1 enzyme activity increased [2.6]. Sulphation is one of a number of liver detoxification pathway, particularly the phase II detoxification. The sulphation detoxification of toxins, it is an antioxidant and detoxification compound produced a powerful agent in the cytoplasm of every cell of the human body [3.13].

Related glutathione antioxidant enzyme involved in the metabolism and detoxification of cytotoxic and carcinogenic compounds as well as reactive oxygen species.

The generation of reactive oxygen species occur in relatively prolonged hypoperfusion conditions such as aging. The etiology of presbycusis is much less certain; However, the genetic cause is most likely complex. The effects of aging shows a variety of inter-wide.

The results showed that each individual has a different cost. This is because each individual has a different effective dose of food. The effective dose can also bedepending on the amount of inhaled benzene concentration, weight, and length of employment. The higher the concentration of benzene in the body, the greater the mass of food needed detox. This is in

accordance with the formula that has been made in previous studies suggesting that he has a synergistic relationship with the concentration of 8 weight, length of employment, and the concentration of benzene can affect the intake of non-carcinogens in each individual that can influence the effective dose of food. This is consistent with previous research that says that the genetic variance, sex, and weight may play a role in the biotransformation enzymes [9].

By knowing the foods that can be used to detoxify benzene exposure of the body, in the paint industry Sidoarjo who have a high risk of exposure to benzene can prevent this is through food. In addition, knowing the estimated costs to be incurred in order to prevent exposure to benzene through this food, workers can choose foods that can detoxify benzene in the body with the food that is in line with workers' earnings in the paint industry Sidoarjo.

Sulphation is one of a number of liver detoxification pathway, particularly the phase II detoxification. Sulphation system is important in detoxification of several drugs, food additives and especially the gut bacteria and toxins from environmental contaminants. Glutathione is an antioxidant and detoxification compound produced a powerful agent in the cytoplasm of every cell of the human body. In broad terms, these studies have found glutathione to protect against oxidative stress, detoxification of chemicals and toxins, improve immune function, and support healthy aging. One of the toxins that can be detoxified is benzene.

The results showed that each individual has different needs detoxification intake. This is because each individual has a different effective dose of food. The effective dose can also depend on the amount of inhaled benzene concentration, weight, and length of employment. The higher the concentration of benzene in the body, the greater the mass of food needed detox. This is in accordance with the formula that has been made in previous studies suggesting that he has a synergistic relationship with the concentration of a substance. Weight, length of employment, and the concentration of benzene can affect the intake of non-carcinogens in each individual that can influence the effective dose of food. This is consistent with previous research that says that the genetic variation, sex.

By knowing which foods can be used to detoxify the body's exposure to benzene from, shoe workers who have a high risk of exposure to benzene can prevent this is through food. In addition, knowing the estimated costs to be incurred in order to prevent exposure to benzene through this food, workers can choose foods that can detoxify benzene in the body with the food that is in line with workers' earnings in the paint industry Sidoarjo.

Conclusion

To complete the detoxification benzene required in Phase II enzyme CYP2E1 enzyme necessary, sulfation, and glutathione. CYP2E1-rich foods are beef liver, brain, and salmon; Sulfation rich foods are eggs, chicken sulfation, beef and tuna, and foods rich in glutathione are avocado, asparagus, carrots, tomatoes, oranges, and broccoli. Weight, length of employment, and the concentration of benzene can affect the intake of non-carcinogens in each individual that can influence the effective dose of food. By knowing the estimated costs to be incurred in order to prevent exposure to benzene through this food, workers can choose foods that can detoxify benzene in the body with the food that is in line with workers' earnings in the Paint Industry Sidoarjo.

Consequences of benzene exposure in workers obtained from the use of benzene which is very much needed and important role in many industrial processes. This can negatively impact the health of workers if efforts to control or reduce the exposure is not done well. Karsinogenen effect is one of the harmful effects resulting from exposure to benzene in the workplace, especially in the paint industry.

Therefore, the average benzene in Sidoarjo paint industry was 3.28 mg / m3, the average effective dose should be consumed by the respondents to the intake of avocado is 0,011 mg / hr, chicken intake of 0,026 mg / day, The intake of citrus 0,033 mg / hr, and carrot intake was 0.063 mg / hr.So that to reduce the impact of exposure to benzene into the body of the workers in the paint industry needed feeding benzene detoxification. Feeding is different in each individual adjusted to the concentration of benzene, weight, and duration of action.

Conflict of Interest: The authors declare no conflict of interest.

Source of Funding: The source of this research costs from the self.

Ethical Clearance: The study was approved by the Ethics Committee of the Faculty of Public Health,

University of Airlangga.

References

1. Ates NA. Glutathione S-transferase gene polymorphism in presbycusis. WE:WE National Library of Medicine National Institutes of Health; 2005.
2. Basaran, R., Ozdamar, ED, Eke, B. C. (2012). Study on Optimization of Enzyme Activity of CYP2E1 in C57BL / 6 Mice Brain and Heart. FABAD J.Pharm. Sci, 37 (4) :. 191-196.
3. Central Disease Center (CDC). 2013. Facts about Benzene. Retrieved October 11, 2018, fromhttps://emergency.cdc.gov/agent/benzene/basics/facts.asp
4. Hodges, RE, & Minich, DM (2015). Modulation of Metabolic Detoxification Pathways Using Foods and Food-Derived Component: A Scientific Review of the Clinical Applications. Journal of Nutrition and Metabolism, 2015.
5. Cline, JC (2015). nutritional aspects of detoxification in clinical practice. Altern Ther Health Med, 21 (3), 54-62.
6. Cook, SND. Availability of Oral GlutathioneIs Eliminating Previous studies Held Beliefs?. Natural medicines Journal Research Guide.https://www.naturalmedicinejournal.com/sites/default/files/glutathione_final_digital.pdf, 2017.
7. Ferguson, CS, Tyndale, RF. P450 enzyme in the brain: evidence is emerging of biological significance. Trends Pharmacol. Sci, 2011. 32 :. 708-714.
8. Lewis, DFV. A guide to the structure and function of cytochrome P450. Taylor & Francis, London. 2001.
9. Pietryka, J. sulphation, Detoxification, and ASD.2014.
10. Purdy, M. Why Detox? Tips and Tricks for Patient Guiding you towards a Healthy Heart.https://integrativerd.org/wp-content/uploads/2012/04/Why-Detox-UPDATED-PPT.pdf, 2012.
11. Smith, Martyn T. Advances in Understanding Health Effects of Benzene and Vulnerability. Division of Environmental Health Sciences, School of Public Health, University of California, Berkeley, California. 2010
12. Tualeka, Abdul Rohim. Industrial Toxicology. Mulia Graha Science: Surabaya; , 2013.
13. Bachelor, Septhanie YP *Analysis of Health Risks Of Exposure to Benzene In Full In Part Solvent Base (Case Study Paints Factory, PT. Tunggal Djaja Indah, Sidoarjo).*Thesis thesis, UNIVERSITY AIRLANGGA.
14. Winata, SD. Impact and Monitoring in Workers Exposed to Benzene. Fk Ukrida. 2018.

Salivary Zinc level and Taste Detection Thresholds in Hypertensive Patients on Amlodipine and on Losartan (A Comparative Study)

Yosor M. Akram[1], Taghreed F. Zaidan[2], Zaid M Akram[3], Omar A. Abed Alsattar[4]

[1]M.Sc.student, [2]Professor, Department of Oral Diagnosis, Collage of Dentistry, University of Baghdad, [3]Lecturer, Al-Rafiddain University College, [4]High Diploma Degree in Periodontics, Al-Ameriya Specialized Center for Dentistry, Baghdad Medical Department /AlKarkh , Ministry of health.

Abstract

High blood pressure (hypertension) is one of greatest risk factors for cardiovascular disease,which is a remarkable cause of morbidity and mortality worldwide. The outcome of hypertension (HTN) and cardiovascular disease (CVD) is influenced by a wide variation of risk factors like use of tobacco; excessive alcohol consumption; unhealthy diet, few physical activity ,overweight and obesity; high blood glucose, and abnormal blood lipids. The objectives of this study were to evaluate taste detection thresholds (of four basic tastes) of hypertensive patients on Amlodipine 5 mg and on Losartan 50 mg. And to estimate Zinc level in saliva of those patients and compare it with control subjects. A total of 90 subjects were incorporated in this study they were divided into three groups: 1-Thirty patients on Amlodipine (5mg) 2-Thirty patients on Losartan (50mg) and 3- Thirty healthy control subjects. Unstimulated whole saliva was collected from all subjects including in this study. Volume of 5 ml of each taste gradient solution,was offered to the participants. The samples were subjected to biomechanical analysis to estimate zinc level by using the atomic absorption spectrophotometer.The result showed that the taste detection threshold of sucrose and sault were significantly higher in patients on Amlodipine and on Losartan treatment than in control subjects. While the taste detection threshold of sour and bitter showed no significant differences between the study groups. Also there is un alteration in salivary Zinc, Zinc in patients on Amlodipine and on Losartan was significantly lower compared to control group.

Keywords: *Taste Detection Thresholds, Hypertensive, Amlodipine, Losartan, Zinc*

Introduction

Hypertension is often called a silent killer since frequently there are no clear symptoms. Initial signs of hypertension may be non-specific (headaches, excessive irritability, insomnia, decreased tolerance to exercise, palpitations and flushing of the head, neck and chest) and thus confusing[1]. Hypertension has lately been reaffirmed as the major single risk factor contributing to world death rates[2]. Besides , control of hypertension is one of the most cost-effective process to decrease early cardiovascular morbidity and mortality[3]. The national institute for health and care excellence(NICE) recommended that management of high blood pressure with any of many classes of common medication is cost saving compared with providing of no medication .The aims of management of hypertension is to control arterial pressure, prevent end-organ damage (cardiovascular ,cerebrovascular, and renal), and to decrease the chance of premature death[4].

Classification of drugs may be done by mechanism or site of action (therapeutic british hypertension society, 2014). In each class, there are numerous drugs with variation in structure and pharmacology resulting in alteration in therapeutic and side-effects[5].

Amlodipine is an oral dihydropyridine calcium channel blocker. Compared to nifedipine and other medications in the dihydropyridine class, amlodipine

Corresponding author:-
Yosor Mustafa Akram, general practice dentist in Ministry of health, Baghdad/Iraq ,
E-mail: yosor.m.akram@gmail.com

has the longest half-life at 30 to 50 hours. The advantage of such a long half-life is the ability to have once-daily dosing. Amlodipine (dihydropyridines) has been described to be an efficient antihypertensive medication related with regression of left ventricular hypertrophy and vascular hypertrophy, the antiatherogenic and the remodeling effects[6].

Losartan is an angiotensin II receptor antagonist (AIIRA) with antihypertensive action due mainly to selective blockade of (AT1) receptors and as the consequence reduced the effect of angiotensin II in elevation of blood pressure. Losartan use in the management of hypertension and heart failure , especially in patients who develop cough with the use of Angiotensin converting enzyme (ACE) inhibitors, additionally it is used in patients with left ventricular hypertrophy to reduce the risk of stroke, and also used in the management of diabetic nephropathy, and has been tried in management of myocardial infarction[7]. Peak plasma concentrations of losartan are achieved within one hour of oral administration the half-life of Losartan 1.5–2.5 hours[8].

Taste is an important protective sense, progressed to manage the intake of food and help in the avoidance of poison. Genetic considered as the principal determinants of taste threshold, and taste thresholds do not differ significantly from day to day. This has lead to the idea of "non-tasters" and "supertasters''[9], in which the taste threshold is linked to the haplotype(a combination of alleles for different genes and tend to be inherited together) of specific receptors[10]. Taste disturbances may arise secondary to autoimmune disease, inflammation, imbalance of hormone, nerve-related damage, psychological problems like in anorexia, medication therapy or malignancy; they may also occur as a consequence of natural aging[11]. Zinc is an essential trace element and is found in tissues throughout the body, reaching iron in its relative abundance[12]. The body of human carry about 2g of zinc, approximately 60% of which is found in muscle tissue, 30% in bone and 5% in skin[13].

Aims and Objectives

1- To Evaluate taste detection thresholds (of four basic tastes) of hypertensive patients on Amlodipine (5 mg) and on Losartan (50 mg).

2- To estimate Zinc level in saliva of those patients and compare it with control subjects.

Materials and Method

This case control study was conducted in the period from February 2019 to May 2019. After approval from Ministry of Health and College of Dentistry University of Baghdad by the scientific committee. A total of 90 subjects were incorporated in this study, they were divided into three groups: 1- Thirty patients on Amlodipine 5mg. 2- Thirty patients on Losartan 50mg. 3- thirty healthy control subjects.

Inclusion criteria: All patients presented with hypertension and they were under antihypertensive monotherapy for at least 8 months .The antihypertensive medications that used by the patients are: Amlodipine and Losartan.

Exclusion criteria: hypertensive patient taking combination of antihypertensive medications, Diabetes mellitus, Renal failure, smoking, patients with history of radiotherapy in the head and neck region ,and history of chemotherapeutic treatment in the last 3 months.

Each taste gradient consisted of 15 solution ,from 1.5 to 15.5 mmol (in 1 mmol increments) for sucrose, from 1-78 mmol (in 5.5 mmol increments) for sodium chloride , from 48 -720 µmol (in 48 µmol increments)for citric acid , and from 89-117 mmol (in 2mmol increments) for urea[14]. Taste solution were Prepared by calculation the amount of taste substance in grams dissolved in deionized water for recommended concentration according to weight (g)= molecular weight(g/mole) x concentration(M) x volume(ml)/1000[15]. The concentrations of solutions used are shown in table (1). The sip and spit method was used, the taste solution were swirled around in the mouth briefly and expectorated into an empty cup[16].

Table (1): The concentration of taste solutions used (Amerine and Pangborn, 1965)[14]

Sucrose mmol/L	Sodium chloride mmol/L	Citric acid µmol/L	Urea mmol /L
1.5	1	48	89
2.5	6.5	96	91
3.5	12	144	93
4.5	17.5	192	95
5.5	23	240	97
6.5	28.5	288	99
7.5	34	336	101
8.5	39.5	384	103
9.5	45	432	105
10.5	50.5	480	107
11.5	56	528	109
12.5	61.5	567	111
13.5	67	624	113
14.5	72.5	672	115
15.5	78	720	117

Statistical Analysis

The statistical package for the social sciences (SPSS) was used for data input and analysis. The statistical significance of difference in mean between more than two groups was assessed using the ANOVA model. When statistically significant difference was shown by the use of ANOVA, further exploration of the statistical significance of difference in mean between each two groups was assessed by post –hoc multiple comparison Least Significant Difference(LSD) test.

Results

This study showed that the mean and standard deviation of the taste detection threshold of sucrose (sweetness) of patients on Amlodipine was 14.16± 2.48 mmol/l , and those on Losartan treatment was 12.76±3.20 mmol/l. For the control subjects was 8.16 ±2.48 mmol/l. It has been found that the taste detection threshold of sweet showed significant difference ($p<0.001$) using ANOVA test and was highest among patients on Amlodipine (Table 2).Continuing analysis with (LSD) test showed that the taste detection thresholds of sweetness was significantly higher in patients on Amlodipine and on Losartan treatment than in control

subjects (p<0.00 1) but no significant difference has been found between patients on Losartan and patients on Amlodipine (Table 3).

The mean and standard deviation of the detection threshold of salty taste of patients on Amlodipine patients was 52.90± 12.03 mmol/l , and on Losartan treatment was 55.83± 14.94 mmol/l , while in control subjects was 32.11±21.66 mmol/l. It has been shown that the detection threshold of salt was significantly higher in patients on Amlodipine and Losartan treatment than that in the control subjects (Table 2) .Continuing analysis with (LSD) test, showed that the taste detection thresholds of salt in patients on Amlodipine was significantly higher than that of control subjects. And the taste detection thresholds of salt in patients on Losartan was significantly higher than that of control subjects , but no significant differences was found between patients on Amlodipine and patients on Losartan (Table 3).

For the sour taste, the results showed that the mean and standard deviation of the detection threshold for citric acid in Amlodipine patients was 473.43±183.91 µmol/l and of patients on Losartan treatment was 453.23 ±175.59 µmol/l, while in control subjects the detection threshold for citric acid (sourness) was 515.46±192.34 µmol/l (Table 2). Statistical analysis showed no significant differences between the study groups.

For the bitter taste, it has been shown that the mean and standard deviation of urea (bitterness) in patients on Amlodipine patients was 91.75± 21.09 mmol/l, and for Losartan treatment was 85.00 ± 26.32 mmol/l, while for control subjects was 92.20±17.51 mmol/l (Table 2).No significant differences was found between the study groups.

Table (2):- The mean and standard deviations of the taste detection threshold of the four tastes in study groups

Group	Sweet		Salt		Sour		Bitter	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Amlodipine	14.16	2.48	52.90	12.03	473.43	183.91	91.75	21.09
Losartan	12.76	3.20	55.83	14.94	453.23	175.59	85.00	26.32
Control	8.16	2.48	32.11	21.66	515.46	192.34	92.20	17.51
P. value	0.00** HS		0.00** HS		0.41 NS		0.36 NS	

All units in mmol/L except for sourness in µmol/l

** HS:Highly significant p<0.001 NS: - None significant p> 0.05

Table (3): - Multiple comparisons using LSD among study groups

Variables	Subgroups	Subgroups	Std. Error	P. Vlue
Sweet	Amlodipine	Losartan	.70857	0.051
		Control	.70857	.000 HS
	Losartan	Control	.70857	.000 HS
Salt	Amlodipine	Losartan	4.3147	0.498
		Control	4.3147	.000 HS
	Losartan	Control	4.3147	.000 HS

** HS: Highly significant p<0.001

For salivary zinc the mean and standard deviation of patient on Amlodipine and Losartan treatment and for the control subjects showed in Table 4.The statistical analysis using ANOVA test demonstrated that salivary Zinc showed significant differences ($p<0.001$) between the control group and the two study groups. Continuing analysis with (LSD) test showed that the mean of salivary zinc in patients on Amlodipine and in patients on Losartan was significantly lower compared to control group ($p<0.001$) .while no significant difference between the mean of salivary zinc in patients on Amlodipine and on Losartan ($p> 0.05$)(Table5).

Table (4):-The mean and standard deviations of salivary Zinc in study groups with ANOVA

Group		**Zn µg /dl**
Amilodipine	Mean	3.29
	SD	0.71
Losartan	Mean	3.45
	SD	0.75
Control	Mean	5.93
	SD	1.03
P-value		.000 HS

HS:-Highly significant at $p<0.001$

Table (5): Multiple comparison of Zinc using LSD among the study groups

Variables	**Subgroups**	**Subgroups**	**Std. Error**	**P. Vlue**
Zn	Amlodipine	Losartan	0.2191	0.476
		Control	0.2191	0.000 HS
	Losartan	control	0.2191	0.000 HS

HS:-Highly significant at $p<0.001$

Discussion

Hypertension (HTN or HT), also known as high blood pressure , is define as a long-term medical condition in which arterial blood pressure is elevated persistently[17].

Sweet detection thresholds of sucrose (sweetness) of patients on Amlodipine and those on Losartan treatment were significantly higher than that of control subjects. Salt detection threshold in patients on Amlodipine and on Losartan treatment which were significantly higher than that of control subjects. For the sour detection threshold, it was lower in patients on Amlodipine and Losartan treatments than the control subjects but it did not reach the significant level. For the bitter detection thresholds it showed no significant differences between the study groups. This result was agree with Tsuruoka *et al.*, 2005 ,who found that Losartan induced taste disturbances and they found that the taste disturbance by the Losartan and Perindopril (ACI-groups) medications at the dosages used was similar in quality and quantity. But disagree with Tsuruoka *et al., (*2005) who stated that Losartan-induced taste disturbances appear to be larger for "bitterness" and "sourness" than "salt" and "sweetness"[18]. It has been hypothesized that a prominent underlying pharmacological mechanism, may explain taste disorders as a class effect of Angiotensin receptor blocker (ARBs). Taste receptors are seven-transmembrane domain G protein-coupled receptors. Angiotensin II receptors belong to the same type of receptor .The sweet and bitter receptors on taste cells are coupled with G-proteins, where G-protein coupling and uncoupling results in taste on and off, respectively ARBs are secreted into saliva, binding receptors of taste and thereby distorting the tastes of sweet and bitter ,Salt and sour tastes may be disrupted by ARBs ion channels plugging or obstructing (salt taste via amiloride-sensitive epithelial Na channels, and sour taste via amiloride-sensitive epithelial Na channels and H+-activated cation channels) found on taste cells[19]. This result was disagree with Kim et al .,(2017) who reported that salt-taste thresholds did not significantly differ between the control and hypertension groups[20].

The result showed that salivary Zinc of patients on Amlodipine and for patients on Losartan treatment were highly significantly decreased than that of control subjects. This result agree with (Korean *et al.,* 2005) who found that Losartan treatment result in Zn depletion, mediated by an increase in urinary Zinc excretion[21].

This result was also agree with (Ifor, 1989) who found that there is a decrease in the level of salivary Zinc level in hypertensive patient[22].

Conclusions

Amlodipine and Losartan can affect taste detection thresholds of sweet, salt in such a way that they were detected taste with high concentrations of taste solutions, but sour and bitter taste was not affected. And Zinc in saliva was decreased significantly by treatment with these medication.

Conflict of Interest We declare that we have no conflicts of interest.

Human and Animal Rights All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee.

Informed consent Informed consent was obtained from all individual patients ,including in this study

Source of funding: Self funding

References

1- GEGENHUBER, A.; LENZ, K.Hypertensive emergency and urgence. Herz, 2003, 28.8: 717.

2- LIM, S.S. ,VOS, T., FLAXMAN ,A.D., A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. The lancet, 2012, 380.9859: 2224-2260.

3- ELLIOTT, W. J.; CHILDERS, W. K., Should β blockers no longer be considered first-line therapy for the treatment of essential hypertension without comorbidities?. Current cardiology reports, 2011, 13.6: 507.

4- Nice Guidelines on Hypertension clinical management of primary hypertension in adults. Manchester: National Institute for Health and Clinical Excellence. http://publications.nice.org.uk/hypertension-cg127,2011;(accessed March 20, 2014).

5- Therapeutics British, Hypertension Society. Available from http://www.bhsoc.org/resources/therapeutics/, 2014.

6- JAMALI,S.N.,MASHORI,G.R.,AZMI,A.A: Antihypertensive efficacy of cardioselective Beta-blocker atenolol and amlodipine in essential hyprtensive patients. Pak J Pharmacol, 2009, 2: 49-54.

7- SWEETMAN, S. C. ,Martindale,E.D. :Martindale. Pharmaceutical press, 2009.

8- MUNAFO,A.,CHRISTIAN, Y., NUSSBERGER, J. Drug concentration response relationships in normal volunteers after oral administration of losartan, an angiotensin II receptor antagonist. Clinical Pharmacology & Therapeutics, 1992, 51.5: 513-521.

9- BARTOSHUK, L.M., CATALANOTTO,F., HOFFMAN ,H., LOGAN,H., SNYDER, D.J.. Taste damage (otitis media, tonsillectomy and head and neck cancer), oral sensations and BMI. Physiology & behavior, 2012, 107.4: 516-526..

10- KIM U.K., BRESLIN. P.A.,REED. D, DRAYNA. D., Genetics of human taste perception. Journal of Dental Research, 2004, 83.6: 448-453.

11- BOYCE, Jamie M.; SHONE, G. R. Effects of ageing on smell and taste. Postgraduate medical journal, 2006, 82.966: 239-241.

12- CHRISTIANSON, David W. Structural biology of zinc. In: Advances in protein chemistry. Academic Press, 1991. p. 281-355.

13- BRIONY,T.; BISHOP IN CONJUNCTION, Jacki; BRITISH DIETETIC ASSOCIATION, the Manual of dietetic practice. Emerald Group Publishing Limited, 2007.

14- Amerine MA, Pangborn RM. Principles of sensory evaluation of food. Book 1st edition. New York: Academic Press. 1965; 75105.

15- KOENIG. J., CUI. Y., NIES. A.T., KEPPLER. D.. A novel human organic anion transporting polypeptide localized to the basolateral hepatocyte membrane. American Journal of Physiology-Gastrointestinal and Liver Physiology, 2000, 278.1: G156-G164.

16- MOJET J , JOHANNES , HEIDEMA J , CHRIST-HAZELHOF E .. Taste perception with age: generic or specific losses in threshold sensitivity to the five basic tastes?. Chemical senses, 2001, 26.7: 845-860.

17- Naish J, Court DS. Medical sciences. (2ed.). 2014; 562.

18 Tsuruoka.S, Wakaumi. M., Araki.N., Ioka T, Sugimoto.K., Fujimura .A. Comparative study

of taste disturbance by losartan and perindopril in healthy volunteers. The Journal of Clinical Pharmacology, 2005, 45.11: 1319-1323.

19- Tsuruoka S, Wakaumi M, Nishiki K, Araki N, Harada K, Sugimoto K, Fujimura A.:Subclinical alteration of taste sensitivity induced by candesartan in healthy subjects. British journal of clinical pharmacology, 2004, 57.6: 807-812.

20- Kim.C.Y , Mi-Kyung Ye ,Young Soo Lee., The salt-taste threshold in untreated hypertensive patients. Clinical hypertension, 2017, 23.1: 22..

21- KOREN.M., DISHY.V., ZAIDENSTEIN.R., YONA.O., BERMAN.S, WEISSGARTEN.J., GOLIK.A .,The effect of losartan and losartan/ hydrochlorothiazide fixed-combination on magnesium, zinc, and nitric oxide metabolism in hypertensive patients: a prospective open-label study. American journal of hypertension, 2005, 18.3: 358-363.

22- IFOR. D. CAPEL., EDGAR. P. SPENCER., ANITA. E. DAIVIES., HARRY .N. LEVITT :The assessment of zinc status by the zinc tolerance test in various groups of patients. Clinical biochemistry, 1982, 15.5: 257-260.

Analysis of Relationship between Work Attitudes and Repetitive Activities with Subjective Complaints on Musculoskeletal Disorder in Circular Loom Division workers PT. Kerta Rajasa Raya Sidoarjo Indonesia

Yuly Eka Saputri[1], Y. Denny Ardyanto W[2]

[1]Department of Occupational Health and Safety, Public Health Faculty, Airlangga, University, 60115, Surabaya, East Java, Indonesia; [2] Department of Occupational Health and Safety, Public Health Faculty, Airlangga, University, 60115, Surabaya, East Java, Indonesia

Abstract

The Musculoskeletal System Disorders (MSDs) are one of the most common occupational diseases experienced by workers. This disorder is influenced by several factors, such asrepetitive activity factor and unnatural work attitude factor. The purpose of this study was to analyze the strong relationship between work attitude and repetitive activity with subjective complaints of musculoskeletal disorder in the circular loom division of PT. Kerta Rajasa Raya Sidoarjo, Indonesia.

This study used observational techniques with cross-sectional design. Respondents in this study were workers in the circular loom division of PT. Kerta Rajasa Raya, Sidoarjo, that consisted of 14 people based on the researchers' inclusion criteria. The data were obtained from the results of questionnaires and field observations. Work attitude data were analyzed using OWAS; MSDs subjective complaint data were investigated using NBM checklist sheets; and repetitive activity data were analyzed based on the results of questionnaires, interviews, and observations.

The results showed that the majority of respondents have subjective complaints of musculoskeletal disorder with high severity. The work attitude variable has a very strong correlation with subjective complaints of MSDs, and repetitive activity variable has a strong correlation with subjective complaints of MSDs.

The company are advised to provide training related to safe lifting method, provide information related to MSDs in the form of posters or providing specific material. Companies are also advised to add the expedition personnel to reduce the repetition of lifting activities.

Keywords: *Subjective complaints of MSDs, Work Attitudes, and Repetitive Activities*

Introduction

The Musculoskeletal System Disorders are one of the most common occupational diseases experienced by workers. According to Tarwaka, Musculoskeletal disorder is a complaint in the part of the skeletal muscle that is felt by a person ranging from very mild to very painful complaints(1). MSDs are the biggest cause of absence in almost all parts of Europe(2). According to the Labor Force Survey (LFS), the prevalence of musculoskeletal cases (MSDs) in the year 2016/2017 amounted to 507,000(3).

NIOSH worker health chartbook of musculoskeletal cases (MSDs) shows that manufacturing industry ranks second after the service industry(4). The manufacturing industry is an industry that converts raw materials into a

Corresponding author:
Y.Denny Ardyanto W,
Department of Occupational Health and Safety, Public Health Faculty, Airlangga University, Surabaya, East Java, Indonesia.
Email: denny.ardyanto@fkm.unair.ac.id
Telp. (+6281330061765)

product. In the manufacturing industry, there are many manual handling activities that can cause the emergence of MSDs subjective complaints. Evadarianto found that the majority of manual handling workers as much as 73.34% in the rolling mill section experienced MSDs complaints[5]. Peter Vi in Tarwaka explained that the emergence of MSDs subjective complaints are influenced by several factors such as excessive muscle stretching, repetitive activity, unnatural work attitudes; secondary factors, the combination causes and individual factors[1].

PT. Kerta Rajasa Raya Sidoarjo, Indonesia, is one of the manufacturing industries concerned in producing packaging that still has a lot of manual handling activities such as lifting, pushing, moving, or pulling heavy loads. One division with the most lifting activities is the circular loom division. Workers of the circular loom division regularlylift as much as 40-160 baskets per day. The weight of each basket is 17 kg. In addition, the work attitudes in lifting activities are often unnatural.

Based on the explanation, it means that workers who carry out manual handling activities have the potential to experience MSDs subjective complaints. Therefore the researcheris interested in conducting research related to the analysis of the strong relationship between work attitudes and repetitive activities with subjective complaints of musculoskeletal disorder in the circular loom division workers of PT. Kerta Rajasa Raya Sidoarjo, Indonesia.

The purpose of this study is to analyze thestrong relationship between work attitude and repetitive activity with subjective complaints of musculoskeletal disorder in circular loom division workers of PT. Kerta Rajasa Raya Sidoarjo, Indonesia.

Material and Method

The type of research was observational with a cross-sectional approach. The study population is 14 workers, with the inclusion criterias of workers aged ≥35 years, doing manual handling activities, and do not have a medical history of back pain, pain in the waist, sprains, and HNP (Hernia Nucleus Pulposus). The sample in this study is the total population.

Research variables are work attitudes, repetitive activities and subjective complaints of MSDs. The instruments used in the study are questionnaires, OWAS observation sheet (Ovako Working-Postur Analysis System), and NBM (Nordic Body Map) checklist sheets.

Analysis of the data used in this study is an analysis of the contingency coefficient for nominal scale data and correlation analysis of Spearman's rho'sfor ordinal scale data. The relationship between the dependent and independent variables will be classified according to Sarwono (2006)[6]:

Tabel 1. Koefisien Kontingensi

Contingency Value	Level of Relationship
0	There Is No Correlation Between The Two Variables
>0,00 – 0,25	Very Weak Correlation
>0,26 - 0,50	Medium Correlation
>0,51 – 0,75	Strong Correlation
>0.75 - 0.99	Very Strong Correlation
1	Perfect Correlation

Findings

The circular loom division has three job sections, namely the expedition section which lifts a basket weighing 17 kg as much as 100-160 times, then the operator section of the circular loom that must lift and move the basket weighing 17 kg which is done 30-50 times in a working day, and the maintenance section of the equipment that is responsible for checking and repairing the machine

Table 2. Distribution of Frequency of

Severity of MSDs Subjective Complaints to Workers in the Circular Loom Division of PT Kerta Rajasa Raya Sidoarjo in 2019

MSDs Complaints	Frequency	Percentage (%)
Medium	6	42,9
High	8	57,1
Total	14	100

The data above shows that all respondents experienced MSDs subjective complaints. As many as 57.1% experienced musculoskeletal subjective complaints with a high category and 42.9% of

respondents in the medium category.

Table 3. Frequency Distribution of Risk level of work attitude and repetitive activity in Workers of the Circular Loom Division of PT Kerta Rajasa Raya Sidoarjo in 2019

Variable	Category	n	(%)
work attitude	Medium	3	21,4
	High	4	28,6
	Very High	7	50,0
repetitive activity	10 - <40	6	42.9
	40 - <200	8	57.1

Based on table 3, it was found that 50,0 % of workers in the circular loom division had a very high-risk work attitude. The most repetitive activities carried out are 40 - ≤ 200 times every working day or 8 hours with a percentage of 57.1%.

The results of the analysis carried out between work attitude variables with MSDs subjective complaints give the Spearman Correlation value of 0,876. So, it can be concluded that there is a very strong correlation between work attitudes and subjective complaints of MSDs.The relationship direction is positive, which means that the relationship goes in the same direction or the higher the risk level of the workers'attitude in doing their work, the higher the severity of subjective complaints of MSDs.

On analysis of repetitive activity variables,the closeness value of the relationship is 0.708 which is between the numbers 0.51-0.75.Thus, it can be interpreted that the relationship that occurs is strong. These values also provide information that the relationship between the variables of repetitive activity with subjective complaints of MSDs run in line for positive values. So that,the more repetitive lifting activities performed by workers in a day, then the severity of MSDs subjective complaints will also be higher.

Discussion

Musculoskeletal Disorders (MSDs) are a type of pain complaint that is felt in the skeletal muscle system. The pain is usually emergedin the joints, ligaments, or tendons. Complaints in the musculoskeletal system are usually in the form of chronic complaints that are often felt sometime after the worker does his activities and often leaves traces or residues that continue to be felt the next day. MSDs can be caused or aggravated by various risk factors in the workplace such as work attitudes, repetitive activity, vibration, and temperature [(1)].

Based on the results of research conducted at the circular loom division of PT. Kerta Rajasa Raya Sidoarjo, it was found that the majority of respondents experienced MSDssubjective complaints in the high category. All workers who experienced MSDs subjective complaints were operator workers and expedition workers who both carried out manual handling activities in the form of lifting baskets containing plastic thread rolls. The results of interviews conducted further found that the complaints felt by workers were spread almost throughout the body parts of workers. The location of complaints on the body felt by all workers respondents is the left and right shoulder, left and right upper arm, left and right forearm, and both legs.

Based on the results of the research and data processing of attitude variables with the OWAS method, it is known that all expedition workers have very high category of work attitudes whereas for the operator section 3 people have a medium risk, 4 people have a high risk, and 4 people have a very high risk. This is because the work of lifting in the expedition section is carried out with the body bent and twisted aside forming flexion. Moreover,the position of the hand when taking the basket is above shoulder height with a weight of 17 kg. So that, the value obtained by all expeditions is 4 (very high). This is different from the 3 operator workers who have a medium work attitude, even though the burden to be lifted is the same yet the liftingmethod is different. As many as 3 operators who have medium category of work attitudes are carrying out lifting activities without twisting their bodies so that for the back side get to point 2. Besides, the position of the two arms of the operator is also below the shoulder height so that his working attitude is in the medium category.

The results of the analysis get a very strong relationship between work attitude variables and MSDs subjective complaints experienced by workers. Therefore, the higher the risk of work attitude, MSDs subjective complaints will also increase. The results of interviews and observations were made found thatall workers did not know about MSDs and workattitudes

matters. This was due to the education of workers who were mostly only up to high school. In addition, workers had never received information regarding good and correct work attitudes when doing work from the company. This research is in line with the research conducted by Purwosusilo (2015) which states that there is a significant relationship between work attitudes and subjective complaints of MSDs in traditional sand miners in Srumbung district,Magelang regency[7].

Repetitive activity or repetitive movement is an activity carried out continuously[1]. In the circular loom division of PT. Kerta Rajasa Raya Sidoarjo, the effect of repetitive activity is extremely felt in the expedition section.This is due to the expedition workers who are on averagelifting 100-160 baskets daily. Expedition workers complain of pain in the shoulder, upper arm, forearm, calf, leg, and waist. The expedition section also did a basket arrangement onpallet truck as 5 tall stacks with a twisting body position that caused complaints on the workers' waist.

Muscles complaints can occur when the muscles receiving pressure due to lifting the load continuously without obtaining sufficient relaxation time[1]. This theory is in line with the results of research which state that there is a strong correlation between repetitive activity variables and MSDs subjective complaints. hence, the higher number of repetitions carried out, the subjective complaints MSDs will also increase. This study is not in line with the research conducted by Purwosusilo (2015) in traditional sand miners in Srumbung district, Magelang regency, which states that there is no relationship between the frequency of swinging with complaints on upper limbs; or in other words, there is no relationship between repetitive activity and musculoskeletal disorders.[7]

Conclusion

Based on the explanation above, it can be concluded that:

1. The majority of respondents have subjective complaints of musculoskeletal disorder with high severity.

2. There is a very strong relationship between work attitude variables with subjective complaints of musculoskeletal disorder.

3. There is a strong relationship betweenrepetitive activity variables with subjective complaints of musculoskeletal disorder.

Recommendation

1. The company is advised to provide training regarding how to lift in the right position or attitude to reduce the risk of unnatural work attitudes.

2. The companyis advised to provide informationin the form of posters or work instruction procedures as a workers reminder related to the dangers of MSDs and safe lifting method to reduce the level of MSDs subjective complaints and risk of unnatural work attitude.

3. The company is advised to increase the number of expedition personnel by assisting operator section to reduce repetitive movements by expedition workers.

Conflict of Interest: All authors have no conflicts of interest to declare.

Source of Funding: This is an article "Analisis Faktor Penyebab Keluhan Subjektif Musculoskeletal Disorder (MSDs) (pada Pekerja Shift Pagi Divisi Circular Loom PT. Kerta Rajasa Raya)" of Occupational Health and Safety Department that was supported by Faculty of Public Health, Airlangga University.

Ethical Clearance: The study was approved by the institutional Ethical Board of the Public Health, Airlangga University.

References

1. Tarwaka. Ergonomic Industry: Edisi Kedua. Surakarta: Harapan Press; 2015.
2. LS News. Non Fatal Occupational Injuries And Illnesses Requiring Days Away From Work, 2007; 2008.
3. LFS. LFS - *Labour Force Survey - Self-reported work-related ill health and workplace injuries: Index of LFS tables* .The Office for National Statistics Health and Safety Executive; 2017.
4. NIOSH. *Worker Health Chartbook 2004*. Cloumbia: Department Of Health And Human Services; 2004.
5. Evadarianto, N., Dwiyanti, E. Work Posture with Musculoskeletal Abnormalities in Workers Manual Handling Rolling Mill Parts.*The Indonesian Journal of Occupational Safety and Health.* 2017;6(01):97-106.

6. Jonathan, S. Quantitative and Qualitative Research Methods. Yogyakarta : Graha Ilmu; 2006.
7. Purwosusilo, P., Wahyuni, I., dan Suroto. Relationship between Work Posture and Frequency Swinging with Complaints of Upper Body Members in Traditional Sand Miners in Srumbung District, Magelang Regency.2015; 3(02)

Application of Infection Control Rules by Iraqi Orthodontics

Rehab adil Al-Rawi[1] Noor Nourie Abbass Abdulla[1], Fudhla Sadoon Al- Zubaydi[2], Bassam Alsheekhly[1]

[1]College of Dentistry/ Al –Iraqia university/Iraq, [2]College of Dentistry / University of Baghdad/Iraq

Abstract

Orthodontics represent an important dental speciality which deals different situations of patients malocclusion, they use many kinds of sharps and cutting instruments, making them liable to different types of infections which could be transmitted from either the patients or the instruments if they don't follow the infection control principls. The data were collected by answering a questionnaire by 101 orthodontists, the results showed different responses of the participants to the variables used by the questionnaire leading to a conclusion that more efforts are needed to improve the application of infection control guidelines between Iraqi orthodontists.

Keywords: *orthodontics, infection control, health, patients; infection.*

Introduction

Orthodontists facing various types of microorganisms during practicing dentistry by contaminated instruments, inhalation of aerosols or through percutaneous injuries with different wires such as ligature or arch wires also banding and bonding materials and other sharp instruments(1).

Studies found that Orthodontists have the second highest occurance among dental workers concerning hepatitis–B infections (2). People reciving treatment in dental clinic could be undetected hepatitis-B carriers and patients secreting herpes simplex viruses in saliva may be asymptomatic, those patients have the potential for transmitting diseases. Diseases such as hepatitis-B, HIV and tuberculosis have long incubation period and therfore, it is very difficult to detect the origin of such infections to the dental workers and other patients (3).

Before beginning with work the orthodontist should be clear about his or her goals in infection control criteria and it is mandatory to apply the most progressed method of disinfection and sterilization to get good results(4).

Sterilization kills all types of microorganisms including viruses, bacterial and mycotic spores.

Corresponding author:
Bassam Alsheekhly
E-mail: bassam_rehab@yahoo.com

Disinfection is the technique used for destruction or inhabition of the most pathogenic microorganisms and inactivating some viruses,therfore, reduction of microbial contamination to a safety levels.(5)

Hepatitis B virus can be spreaded by as little as 0.0004 ml blood while HIV by 0.1 ml blood(6). 1 ml of gingival crevicular fluid contains 150 billion microorganisms and 6 billion microorganisms can be found in 1 ml of saliva (7).

Many studies in literature reavels that emphasize on the effect of sterilization in orthodontics practice however there is no comprehensive research that evaluate the compliance of Iraqi orthodontist to infection control procedures.

Aim of the Study

In this study we will evaluate sterilization and disinfection methods employed in orthodontic practice in Iraq.

Material and Method

In the present study, data collection gained by an 17 items questionnaire was delivered to a total of 101 Iraqi orthodontists / general practitioners(GP) (who attended intensive orthodontic course), these question covered some infection control guide line.(8)

Statistical analysis was performed by using SPSS version which includes descriptive statistic (frequency

and percent) also Z-test were used to test two proportion and chi square for goodness of fit to test more than two proportion. N.S non-significant (P> 0.05), S significant at (P<0.01) and highly significant (P<0.001).

The questionnaire deals with the following variables:

- Educational degree
- Place of work.
- Daily patient volume
- The way in performance of instruments cleaning
- Sterilization devices used
- Soaking instruments in disinfectant solution.
- Packaging method of instruments to be sterilized.
- Method used to sterile hand piece, hand instruments and orthodontic pliers.
- Does the practitioner sterilize molar bands after purchase?
- Sterilization of molar bands after trail inside patient mouth.
- Disposal of brackets, bands and arch wires removed from the patients
- Recycling brackets or other orthodontic materials.
- Disinfectios of the impressions and orthodontic appliances delivered to the lab.
- Place of sharp objects disposal container.
- Type of gloves used during cleaning instruments, and environmental cleaning.
- Hepatitis B vaccination

Result

The result of this study shown in the frequency table explained as the following: table 1 describes the percent of GP and specialist who are alligated to the questioner, (22.8% and 77.2%)for GP &specialist respectivlly.

Table 2 showed that (45.5%,38% and 15.8 %) of the total participate work in privet clinic, hospital and at university clinic, those participate have daily patients volume explained in table 3.

Table 4 show (57.4%) of orthodontist who use manual cleaning significantly higher than ultrasonic cleaner (42.6 %), while highly significant (87%) with autoclave rather than oven and glass bead sterilizer, also highly significant regarding presoaked instrumentand wraping the instrument ,while sterilization of dental hand piece in autoclave (41.6%) was significantly lower than wiping the outer surfaces with (57.4%).

Table 5 show highly significant rate of sterilize pliers (80.2%) than dry heat, while non-significant sterilize band after purchase in comper with highly significant (67.3%) for band after trial in patient's mouth .The disposing of band, bracket and arch wire was highly significant with (49.5%) regarding waste basket .

Table 6 show high rate of significantly for orthodontist who don't recycled bracket while non-significant difference between orthodontist who disinfect impression or appliances . The rate for placing sharp bins at clinic was significant (74.3%) and (21.8%) for placing sharp bins in sterilization room. Highly significant rate for orthodontists who use examination gloves during cleaning of the instrument, and how recive hepatitis B vaccine.

Table 1 Gp or Specialist

Frequency			**Percent**
Valid	GP	23	22.8
	Specialist	78	77.2
	Total	101	100.0

Working place Table 2

Frequency			**Percent**
Valid	Private dental clinic	46	45.5
	Specielized dental centers / state hospital	39	38.6
	University clinic	16	15.8
	Total	101	100.0

Table 3: Number of patient

Frequency			**Percent**
Valid	0-5	40	39.6
	6-10	18	17.8
	11-15	14	14.9
	16-20	15	14.9
	> 20	13	12.9
	Total	100	99.0
Total		101	100.0

Table 4: Percentage of cleaning and sterilization of the instrument

Question	**Choice**	**Frequency**	**%**	**P-value¥**
Cleaning performance	Manually	58	57.4	0.033
	Mechanically (Ultrasonic cleaner	43	42.6	
Sterilization devices used	Autoclave	88	87.1	0.001
	Dry heat (oven)	12	11.9	
	Glass bead sterilizer	0	0.0	
	none	1	1.0	

Cont.... **Table 4: Percentage of cleaning and sterilization of the instrument**

Theinstruments are presoaked in disinfectant solution	Yes	86	85.1	0.001
	No	15	14.9	
Instruments Packing during sterilization	Metal tray	38	37.6	0.021
	Wrpa (Pouching)	41	40.6	
	I do not pack	20	19.8	
Sterilization of dental hand pieces	In the autoclave	42	41.6	0.022
	Wiping the outer surface with disinfection solution	58	57.4	

N.S, not significant (P >0.05) , * significant at (P < 0.05), ** significant at (P < 0.01), * significant at (P < 0.001).**

Table 5: percentage of sterilization of orthodontic pliers, bands and disposed method

Question	**Choice**	**Frequency**	**%**	**P-value¥**
Sterilization of hand instruments / or orthodontic pliers	Dry heat (oven)	12	11.9	0.001
	Autoclave	81	80.2	
	Glass bead sterilizer	1	1.0	
	Wiping with a disinfictant solution	6	5.9	
Sterilization of molar bands after purchase	Yes	48	47.5	0.617
	No	53	52.5	
Steriliztion of molar bands after check in the patient mouth	Dry heat (oven)	10	9.9	0.001
	Autoclave	68	67.3	
	glass bead sterilizer	2	2.0	
	Sitting in disinfectant solution	17	16.8	
Where do you dispose the bands , brackets, and arch wires you remove from patient during or after treatment	Waste basket	50	49.5	0.001
	Sharp bin	27	26.7	
	Metal waste bin	6	5.9	
	Infected waste bin	17	16.8	

N.S, not significant (P >0.05) , * significant at (P < 0.05), ** significant at (P < 0.01), * significant at (P < 0.001).**

Table 6: the response to different question related to infection control roles

Question	Choice	Frequency	%	P-value¥
Recycled brackets or other orthodontic materials	Yes	9	8.9	0.001
	No	91	90.1	
Disinfection of impressions or other orthodontic appliances to be delivered to lab	Yes	48	47.5	0.670
	No	51	50.5	
Where do you place sharp bins	at the clinic	75	74.3	0.001
	In the sterilization room	22	21.8	
Type of gloves used during cleaning of instruments and environmental cleaning	Examination gloves	97	96.0	0.001
	Kitchen - type gloves	3	3.0	
Have you had hepatitis B vaccine	Yes	83	82.2	0.001
	No	16	15.8	

N.S, not significant (P >0.05) , * significant at (P < 0.05), ** significant at (P < 0.01), * significant at (P < 0.001).**

Discussion

Spaulding system classifies instrument into three categories which are critical, semicritical and least critical[9] , the semicritical considered the most important one that should be highlighted in order to prevent disease transmission [10].Orthodontic instruments,orthodontic supplies and accessories considered as the semicritical since they touch mucous membrane and non-intact skin [11,12].

This study mainly depended on experience, place of work as well as, daily patient capacity of the GP and orthodontist. The sterilization process is important to orthodontists as well as, dentists, even though they do not perform surgical procedures [13-14] .

In another hand Starnbach & Akçam [15-16] indicate, sterilization is less abidance to orthodontists than dentists since they usually deal with children, with loss of time , money and the corrosion of orthodontic instruments in addition they did not deal with deep tissues.[8]

Professional agencies like Center of Disease Control (CDC) and Occupational Safety and Health Administration (OSHA), have a specific recommendations representing standard infection control which considered as keywords to be followed in order to prevent cross infection [17].

Despite of these rules, this study showed that (57.4%) of participate orthodontists depends on the manual cleaning procedure more significantly than the ultra-sonic devises. This may be due to the lack of knowledge about advantage of ultra-sonic devises in the granting the proper removing of debris from the orthodontic instrument [18-19], the manual cleaning is also an important step in ensuring the debris removal after mechanical cleaning and before autoclaving [20].

In this study (85.1%) of participated presoaked the instrument in disinfected solution before starting the sterilization process. This prevents the dryness and adherence of bioburden to instruments that protect microorganism from sterilization [21].

In addition to that it begins to dissolve organic debris and in some instances begin microbial kill.This solution should discard at least once a day [17].

This study showed high percentage of participates who used autoclave whether used for orthodontic

pliers , band purches and molar bands after trying in patient mouth [22]. This concurred with the significant findings of participates who pouch their instruments. This could be explained by the high education level and interest in sterilization of orthodontists and according to recommendations of Iraqi Dental Association (IDA).

Although most of orthodontist (87%) using autoclave but the result showed that high percentage of them only wipes the hand piece which is not recommended as the hand piece represents a hallow instrument that may contain blood drops contamination inside the internal lumen and this can be only sterilize by using autoclave class B [23, 24-25-26] .This could be the result of old believes that heat could ruin the hand piece leading to a financial lost.

As a matter of fact the hand piece sterilization is obligatory according to the CDC guidelines. This can be achieved neither by providing clinics with an enough amount of hand piece in order to match the number of patients who daily visit the clinics, nor by applying advanced sterilization programs [27]. Since hand pieces are available in the Iraqi market with an affordable cost.

Bracket, wire and bands represents a dangerous source since they are removed from oral cavity in which they had been contaminated with body fluid (saliva and blood) also wire's end considered as a sharp end that may prick the orthodontist, so the sharp pin represent the better choice for disposing [28,29] .

This study showed that (74.3%) of participant have sharp bin in their clinics, despite of the importance value of having it in the clinics, only (26.7%) who are really using it while (49.5%) of the participant use waste basket. This approved a week point in disposal.

This study showed a highly significant percentage (90.1%) of the participant who do not use recycle brackets and orthodontic materials since the process of recycling altars the mechanical and physical properties also it is nor granted that they are not contaminated[30,31] also, the brackets and orthodontic materials are available in the Iraqi market with a reasonable cost.

In this study, a non-significant value (50.5% -47.5%) have been shown between the participates who disinfected their impression or appliances to be delivered to an outer laboratory. Standers showed that all impression and model must be disinfected before delivering to the laboratory and vice versa [32].Now a day, this complicated issue has been solved by introducing the digital scan in the dental filed [33].

Kitchen gloves considered as a heavy duty gloves that protect the operator from accidental puncher by sharp dental tools and cross infection [17]. Unfortunately,this study showed a high rating of (96%) who use examination gloves which considered a thinner and easy tearing gloves as compared to the kitchen gloves [8].

Dentists and assistances are always mandatory to be vaccinated against hepatitis B virus [34], this coincide with the result of this study which revealed the highly significant rating (82.2%) who had been vaccinated.

Conclusion

The result of this study reveals good behaviours by Iraqi orthodontist for most of infection control steps although some behaviors need to be improved following world wide infection control guide lines.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None.

Funding: Self-funding

References

1. Bagramian RA, McNamara JA Jr. A prospective survey of percutaneous injuries in orthodontists. Am J Orthod Dentofac Orthop 1998;114:654-8.
2. Rohit D, Sudhir M, Amandeep K, Amanpreet Singh N. Sterilization in orthodontics – A review. Int J Curr Res 2016;9:39056-9.
3. Kiem RG (2012). Remaining Vigilant. J. Clin. Orthod. 46. 521522.
4. Smith GE. Glass bead sterilization of orthodontic bands. Am J Orthod Dentofacial Orthop 1986;90:243-9.
5. Saniç A. Sterilization Applications and Problems in Turkey. Clinics J Microbiol Infec 2003;2:45-58.
6. Kumar V, Mogra S, Shetty SV. Hepatitis B: Facts and concerns to Orthodontist in India. J Ind Orthod Society 2007;41:30-4.
7. Checchi L, Matarasso S, Pirro P, D'Achille C. Topographical analysis of facial areas most susceptible to infection with transmissible diseases

in dentists. Int J Periodontics Restorative Dent 1991;11:164-72.

8. Gümrü Çelikel AD, Ekmekçioğlu H, Külekçi G, Fıratlı S. Evaluation of the Compliance of the Orthodontists to Infection Control Procedures in Turkey. Turk J Orthod 2018; 31: 37-49.
9. Spaulding EH. Chemical disinfection of medical and surgical materials. In:Lawrence C, Block SS, editors. Disinfection, sterilization, and prevention. Philadelphia: Lea & Febiger(1968); 517-531.
10. Rutala WA, Weber DJ. Orthodontic instruments and supplies: Are they semicritical or critical items? Am J of infection control 2017; 45:210-3.
11. Bhatnagar S. Bagga DK. Sharma P, Kumar P. Sharma R, Singh V. Infection control strategy in orthodontic office. European J Gen Dent 2013;2:1-7.
12. de Almeida FCM. de Carvalho AS. Duarte DAL Evaluation of disinfection methods *of* orthodontic pliers. *Dental* Press J Orthod 2012:17:105-9.
13. Shailer P. Clinical Dental Hygiene. The CV Mosby Company. 4th ed. St. Louis, 1972.
14. Bhatnagar S, Bagga DK, Sharma P, Kumar P, Sharma R. Infection control strategy in orthodontic office. Eur J Gen Dentistry 2013; 2:1 -7.
15. Starnbach H, Biddle P. A pragmathic approach to asepsis in the orthodontic office. Angle Orthod 1980; 50:63-6.
16. Akcam M, Ozdiler E. Ortodontide Sterilizasyon ve Dezenfeksiyon. Cumhuriyet Oniversitesi Di$ Hekimligi Fakultesi Dergisi 1999; 2: 129-33.
17. Shilpa K,Tulika T, Priyank R. Infection control in Orthodontics. Journal of Orthodontic and Endodontics 2015;1 No.1:1
18. Mohiuddin S, Dawam N. knowledge, attitude and practice of infection control measures among dental practitioners in public setup of Karachi, Pakistan: cross sectionsurvey.2015,9(1): 1-2.
19. Almaweri S.Tarakji B, Addin B, Alshamiri H, Alaizari, Almasri O. lnfectio control: knowledge and compliance among Saudi undergraduate dental students.GSM Hyg Infection control.2015,10:DOC 10.
20. Jorgensen G, Palenik CJ. Instrument sterilization. Dent Equip and Materials. 2004,9:69-71.
21. Bassam A., Sulafa E. and Rehab A. Awarness and application of infection control recommendations in the private practice of Iraqi dentists.
22. Benson P.E., Douglas C.W.I. Decontamination of Orthodontic Bands following Size Determination and Cleaning. Journal of Orthodontics, 2007; 34:18-24.
23. Cleveland JL, Foster M, Barker L, Brown G, Lenfestey N, Lux L, Corley TL, Bonito AJ. Advancing infection control in dental care settings: factors associated with dentists' implementation of guidelines from the Centers for Disease Control and Prevention. J Am Dent Assoc 2012; 143:1127-38. [CrossRef]
24. Kangane SK. Instrument sterilization in the orthodontic clinic: A review. *Int J Clin Dent Sci.* 2011; 1(1).
25. Reddy VP, Reddy GY, Hegde N, Priyadarshini A. Sterilization Methods in Orthod ontics-A Review. *Int J Dent Clin.* 2011;3(1).
26. George O.RapinC, Benoit F.Filleul MP. Study of corrosion and wear of surgical instruments during sterilization. *Bull Group IntRech Sci Stom- atol OdontoL* 2011;50(2)39-41. [PubMed: 22750710].
27. Centers for Disease Control (CDC). Guidelines for Infection Control in Dental Health-Care Setting. MMWR 2003; 52:1-66.
28. Belstrom D, Paster BJ, Fiehn NE, Bardow A, Holmstrup P. Salivary bacterial fingerprints of established oral disease revealed by the Human Oral Microbe Identification using Next Generation Sequencing (HOMINGS) technique. / Oral Microbiol. 2016;8:30170. doi: 10.3402/jom. v830170. [PubMed: 26782357].
29. Grassl N, Kulak NA, Pichler G, Geyer PE, Jung J, Schubert S, et al. Ultra- deep and quantitative saliva proteome reveals dynamics of the oral microbiome. Genome Med. 2016;8(1):44. doi: 10.1186/sl3073-0i6-0293- 0. [PubMed: 27102203].
30. Bavikati VN, Singaraju GS, Mandava P, Killamsetty SS, Nettam V, Kar-nati PKR. Evaluation of Mechanical and Physical Properties of Clinically Used and Recycled Superelastic NiTi Wires. *J Clin Diagnos Res.* 2016;10(7):ZC35.
31. Oshagh M, Hematiyan MR, Mohandes Y, Oshagh MR, Pishbin L. Autoclaving and clinical recycling: Effects on mechanical properties of orthodontic wires. Indian J Dent Res 2012; 23: 638-42. [CrossRef]

32. *Incognito.* Incognito Lab order farm. TOP-Service fur Lingualtechnik GmbH. *2016. Available from:* www.incognito.net.

33. Dawood A. Marti Marti B, Sauret-Jackson V, Darwood A. 3D printing in dentistry. *Br Dent J.* 2015;219(ll):521-.doi:10.1038/s).bdj.20l5.914. | PubMed: 26657435].

34. Kocabas E. Hepatit B Hastahgi ve A§ilari, Qikurova Universitesi Tip Fakiiltesi Qocuk Enfeksiyon Hastaliklari Bilim Dali, Adana, 2004.

Mast cell, IL-1 Beta and IL-6 for Wound timing and Vitality in Forensic Practice

Mohammad Abdul Mohsin Jebur[1], Nabeel Ghazi Hashim[2], Salim R. Hamoudi Al-Obeidi[3]

[1]Al-Mustansiriyah University/College of Medicine- Department of Pathology and Forensic Medicine, [2]Baghdad University/College of Medicine - Department of Pathology and forensic medicine, [3]Baghdad University/College of Medicine - Department of Pathology and Forensic

Abstract

the application of immunohistochemistry has opened a new era for examining the age of the wound by forensic specialists, The aim of our study was to illustrate the use of immunohistochemical marker of mast cells activation (mast cell tryptase "MCT") as a reliable and promising factor of wounds vitality and to Identify the role of pro-inflammatory cytokines: IL 1 beta and IL 6 as parameters of wound age determination. The study was done in the Medico-Legal Directorate of Forensic Medicine (MLD) in Baghdad. The specimens were sera and skin tissues for immunohistochemistry examination, and staining procedures and immunohistochemistry (MCT) ELISA sandwich plate will measure the levels of interleukins. Total autopsy cases were 88 and divided into study group (SG) and control group (CG) .We found that density of Mast cells in sample group was significantly high and higher infiltration that correlates with time passing in the study group as compared by other group in dermis of the sample and control lesions and positive relationship between levels of IL 1-beta & IL-6 level and time progress of wound..

Key words: *forensic, pathology, wound, vitality, IL 1_beta, IL_6 level, Mast cells*

Introduction

During the inflammatory phase, a variety of chemicals are released at the affected site, leading to the recruitment of inflammatory cells, such as neutrophils and plaques Forensic scientists, unlike general pathologists, tend to focus on chronic mapping of the appearance and disappearance of inflammatory cells or substances produced during the inflammatory process[1]. These phenomena - for example, the proportion of positive cells, the level of tissue fibrosis, the distance between inflammatory cells and the free vessels that affected by the degree of injury, which affect the accuracy of the determination of the age of the wound. It is therefore necessary to establish models with varying degrees of injury and to evaluate the parameters involved in wound healing to determine the time of injury[2].

Thus, MCs have been involved in causing many chronic allergic / inflammatory disorders, autoimmune diseases, and cancers. The contributions of MCs in these conditions are the subject of continuous assessment[3]. Acute direct events, the allergic process involves subsequent stages characterized by leukocytes infiltration and initiation of an acquired immune response, followed by a chronic phase involving persistent inflammation, tissue reformation and fibrosis. Thus, the role of MCs at these different stages has gained increasing importance. The dissociation of MCs, in addition to their well-established and widely studied role in IgE-mediated interactions, has been the focus of MCs research in the past decades. However, the determination of the functions of MCs may progress slowly due to difficulties in accessing these cells in vivo and the obstacles encountered when obtained through enzymatic dispersion of tissues or by culture of predators of MCs isolated from the bone marrow or cord or peripheral blood. The culture of predators of mast cells produces a small number of MCs that are often expensive and time-consuming and lead to the emergence of changing phenotypes due to cultural conditions[4].

Several studies reported high levels of IL-1β production by nuclear monocyte, platelet and nuclei cells from active colon lesions in IBD (inflammatory bowel disease) patients. Since IL-1RA levels are only moderately controlled in the colon in patients with IBD, the IL-1RA ratio to IL-1β decreases significantly,

enhancing intestinal inflammation[5]. IL-1β is initially produced as zymogen, but pro-IL-1β does not contain any known functional properties. IL-1β copies are tightly regulated, stimulated by stimulation by TIR or IL-1R stimulation (the first signal required to produce IL-1β) As previously described, IL-1β precursors are bisected in its active form by caspase-1, after inflammatory activation with the NLR (NOD like receptor: NOD for nucleotide-binding domain, Lucien rich repeat containing receptors) pathway (signal 2) [6]. The secretion mechanisms of IL-1β and IL-18 in extracellular space are unclear, but proposed pathways include exocytosis of secretory lysosomes, shedding of membrane micro-vesicles or exososomes, or transfer by membrane vectors, such as ASC (alanine serine cysteine amino-acids transport system) transporters[7].

IL-1β practices a wide range of systemic and local effects. Once in circulation, IL-1β can promote the synthesis of ring-2 oxidation enzymes (COX2) in the vascular network, which stimulates the production of prostaglandin E2 in the brain and fever-mediated. IL-1β also promotes the synthesis of acute phase proteins by liver cells, derives differentiation of macrophages, differentiation of neutrophils and mobilization in bone marrow. Furthermore, IL-1β promotes the recruitment of immune cells to inflammatory sites, by stimulating the expression of adhesion molecules and chemical attractors via endothelial cells[8].

On the other hand Given the IL-6 behavior of restoring the host to the parity state, it is clear that IL-6 acts to control the response of tissue inflammation. In chronic diseases, which are usually manifested by immune stress factors such as intracellular infection and chronic tumors, IL-6 acts not only as a catalyst for acute phase reactions but also as an important player in cellular immune response responses to infected cells and mucosal- The inflammatory reaction is acute, resulting in the destruction of a harmful agent within a short period of time and in a localized area, stimulating an immune response.[8]

IL-6 not only stimulates acute phase reactions but also develops cellular immune responses, including end-stage B cell differentiation, immune globulin secretion and T-cell activation. The main key from acute inflammation to chronic inflammation is the recruitment of monsters to the inflammation area. IL-6 is important for the transition between acute and chronic inflammation. IL-6 plays a somewhat unexpected role in recruiting white cells in vivo. The IL-6 and sIL-6Rα compounds can activate endothelial cells to secrete IL-8 protein and MCP -1, and urge the expression of adhesion molecules[9].

The aim of the study is to illustrate the use of immunohistochemical marker of mast cells activation (mast cell tryptase "MCT") as a reliable and promising factor of wounds vitality and to identify the role of pro-inflammatory cytokines: IL 1 beta and IL 6 as parameters of wound age determination.

Methodology

The study was done in the Medico-Legal Directorate of Forensic Medicine (MLD) in Baghdad. Lacerated skin wounds with a known timing since injury less than 12 hours, regarding time of autopsy all cases were underwent autopsy as soon as possible within a maximum time of 3 hours since arrival to mortality. Only frank lacerated non-contaminated skin wounds were taken in account and the autopsy was done immediately on arrival to MLD. Total autopsy cases were 88 and divided into study group (SG) and control group (CG) [stab-wounds injuries with immediate death]. The specimens are sera and skin tissues for MCT immunohistochemistry.

ELISA sandwich plate will measure the levels of interleukins and specimens for histopathology will be taken from the periphery of wounds for SG and an intact healthy skin from same cadaver (internal control) and some tissue specimens from deaths due immediate death by bullet injury as (external control), stored in 10% formalin for staining procedures and immunohistochemistry (MCT) and the results was analyzed statistically.

Strict exclusion criteria for cases of study include mixed wounds, wounds with unknown timing since injury, contaminated lacerated wounds, wounds with expected timing less than 12 hours, firearm deaths, cases with a known documented history of chronic illnesses, age less than 15 and more than 35 years, pregnant, decomposed cadavers (delayed autopsy),major medical interventions like surgery.

The Quantikine® Human IL-1β/IL-1F2 Immunoassay is a 3.5-4.5 hour solid phase ELISA designed to measure human IL-1β in cell culture supernates, serum, and plasma The Quantikine® Human IL-6 Immunoassay is a 4.5 hour solid phase. Both contain E. coli-expressed recombinant human IL-1β and IL 6

and antibodies raised against the recombinant factors.

TB staining was used for presence of "Mast cells" and MCT immunohistochemistry by Elabsceince® is the preferable way for detecting mast cells activation.

Results

The number of wounds in the study sample was determined; most of the bodies had three wounds, 44% of the total study, where 6% of the cases had only one wound. The study group of 156 laceration skin wounds was classified into compression laceration (50%), grinding laceration (20.5%), cut laceration (6.5%), tearing (15.3%) and crush injuries (7.7%)

Mast cells by toluidine blue stain

Mast cells are found in connective tissue and the cytoplasm contains **(heterogeneous)** granules consisting of **heparin and histamine**. Toluidine blue stains mast cells with red-purple color **(metachromatic staining)** and blue background **(orthochromatic staining)** Metachromasia and tissue color staining elements differ according to the features of the dye solution due to the pH, dye concentration and temperature of the underlying dye. Blue or purple dyes will show a red shift while red dyes will show a yellow shift with contrasting tissue elements.

Depending on the number of mast cells stained by toluidine blue, three classes of were estimated, from 2 to 5 cells on 10 HPF the number of mast cells stained was regarded **normal finding**, from 6-8 cells on 10 HPF (+1) and from 9-10 cells on 10 HPF and (+2) and > 10 cells on 10 HPF (+3) The specimens included in the study did not have any histologic abnormality. Using light microscopy, all the mast cells (MC) in the specimens (however fixed) had meta-chromatically stained purple with toluidine blue. The nuclei of all the cells were round or oval. MC were scattered in the dermis, especially along the blood vessels and in the peri-glandular stroma. In the dermis of the Sample and control lesions, Mast cell density in the Sample group was significantly higher ($P<0.001$) when compared with the other group.

Table 1: Distribution of mast cells

Immunohistochemistry (Mast cells tryptase) for both samples and control

Immunohistochemistry of Mast Cell Tryptase results were recognized and classified into 4 classes according to the mast cells in both control and samples through the period of 6 hours after injury. Tryptase immune reactivity was detected in all of the cells stained by fluorescent avidin fixed either with Bouin or Carnoy's fluid. Some MC, in the dermis of all the examined groups, was immune stained for chymase upon fixation with Carnoy's fluid.

Table 2 correlation between test group and control group number of mast cells

ELISA levels of IL-1 beta and IL-6 in the human model

IL-1 beta standard of the kit measures at 3.9 pg/ml and minimum detectable dose (MDD) of human IL-1 beta is less than 1 pg/ml. The levels of IL-1 beta in the human model shows a significant decrease in relation to the time of the test (1 to 6 hours AMI), as showed in table 2 that the levels of IL-1 beta in the human model samples shows a maximum mean difference of 40.727 at 1 hour of AMI while the control group mean difference was 284.584. The mean difference decreased by the increasing of time after AMI to reach 3.49 after six hours. The study results suggested a reverse relationship between AMI time and the levels of IL-1 beta in the human model.

Table 3: The levels of IL-1 beta in the *h*uman *m*odel

H1, after one hour AMI H4, after four hours AMI c, control

H2, after two hours AMI H5, after five hours AMI

H3, after three hours AMI H6, after six hours AMI

IL-6:

IL-6 standard of the kit measures at 3.13 pg/ml and the MDD of human IL-6 is typically less than 0.70 pg/ml. The IL-6 levels show **increase** with the time of **AMI** progress. The control samples show mean difference of .57436. The sample group shows maximum mean difference of IL-6 level of 353 at five hours of AMI.

Table 4 : The levels of IL-6 in the human model

One-Sample Test						
	Test Value = 0					
	t	df	Sig.	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
c	5.980	10	.000	.57436	.3603	.7884
h1	1.127	10	.286	269.933	263.73	803.59
h2	15.359	7	.000	2.16250	1.8296	2.4954
h3	13.272	9	.000	1.40290	1.1638	1.6420
h4	9.805	5	.000	1.89883	1.4010	2.3966
h5	1.006	3	.389	353.03625	763.9650	1470.0375
h6	6.926	4	.002	.63980	.3833	.8963

H1, after one hour AMI H4, after four hours AMI c, control

H2, after two hours AMI H5, after five hours AMI

H3, after three hours AMI H6, after six hours AMI

Discussion

In this study we found that density of Mast cells in sample group was significantly high (P<0.001) as compared by other group in dermis of the sample and control lesions. This result goes with the results found by Bonilli et al. [10] in the contrary to[11] who noted no significantly different mast cells' number of in normal cutaneous tissue & those in wounds of the sampling which could be explained partly by different techniques & morph-metrical methodology.

In this study we found higher infiltration with mast cells that correlates with time passing in the study group which agrees with the results found by [10] who utilized anti-tryptase & chymase antibodies or avidin to assess density of mast cells in skin wounds through immunofluorescences And found that the MC number in the dermis showed progressive increase within little hours after injury (top at 1 to 3 hours) Matching results was found by [12] who noted a powerful extra-expression of tryptase located in interstitium.

Levels of IL-1 beta in the human model samples show a maximum mean difference of 40.727 at 1 hour of AMI while in control group, the mean difference was 284.584. The mean difference decreased by the increasing of time after AMI to reach 3.49 after six hours .The study results suggested a reverse relationship between AMI time and the levels of IL-1 beta in the human model. This results matches with [13]who found IL-1β, expressed in normal human skin modified in a significant way in vital injuries in epidermal strata, sub-epidermal cellular, vascular and glandular (sweat) and had promoted expression after fifteen and twenty minutes at early increase of reactivity of the epidermis & following thirty to sixty minutes, marked expression was noted, remained many hours & later decreased to a base level again. He commented that it can help as a beneficial method to estimate vitality and aging of wounds, especially during early post-traumatic period before the leukocytic reactions. On another hand this is against what was found by [14] who stated that no wound groups showed a high increase in IL-1b level in comparison to control group. The difference was not significant from statistical view.

In our study the IL-6 levels shows increase with the time of AMI progress which matches with what was found by [15] and the same results was obtained by[16]

Conclusion

Mast cells by toluidine blue stain density in the sample group was significantly higher when compared with the other group in the dermis of the sample and control lesions. The same was shown by Immunohistochemistry (Mast cells tryptase) as there were higher infiltration with mast cells that correlates with time passing in the study group. Regarding ELISA levels of IL-1 beta and IL-6 in the human model the study found a reverse relationship between AMI time and the levels of IL-1 beta in the human model while the IL-6 levels shows increase with the time of AMI progress.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

References

1. Gist, S., et al., Wound care in the geriatric client. Clinical interventions in aging, 2009. 4: p. 269.
2. Das, J., Injury and Wound Healing, in Trauma in Otolaryngology. 2018, Springer. p. 31-52.
3. Ji, X.-Y., et al., Detection of RAGE expression and its application to diabetic wound age estimation. 2017. 131(3): p. 691-698.
4. Dettmeyer, R.B., Vitality, injury age, determination of skin wound age, and fracture age, in Forensic Histopathology. 2018, Springer. p. 241-263.
5. Casse, J.-M., et al., Skin wounds vitality markers in forensic pathology: An updated review. 2016. 56(2): p. 128-137.
6. Renner, R., C.J.C.W.C.M. Erfurt-Berge, and Research, Depression and quality of life in patients with chronic wounds: ways to measure their influence and their effect on daily life. 2017. 4: p. 143.
7. Birincioğlu, İ., et al., Determination of skin wound age by using cytokines as potential markers. 2016. 44: p. 14-19.
8. Mehanna, R.A., et al., The effect of bone marrow-derived mesenchymal stem cells and their conditioned media topically delivered in fibrin glue on chronic wound healing in rats. 2015. 2015.
9. Wahabzada, M., et al., Monitoring wound healing in a 3D wound model by hyperspectral imaging and efficient clustering. 2017. 12(12): p. e0186425.
10. Bonelli, A., S. Bacci, and G. Norelli, Affinity cytochemistry analysis of mast cells in skin lesions: a possible tool to assess the timing of lesions after death. International journal of legal medicine, 2003. 117(6): p. 331-334.
11. Zhong, F.C. and Z.J. Zhen, Localization and quantification of histamine in injured skin as parameters for the timing of wounds. Forensic science international, 1991. 51(2): p. 163-171.
12. Turillazzi, E., et al., Tryptase, CD15 and IL-15 as reliable markers for the determination of soft and hard ligature marks vitality. Histology and histopathology, 2010.
13. Grellner, W., Time-dependent immunohistochemical detection of proinflammatory cytokines (IL-1β, IL-6, TNF-α) in human skin wounds. Forensic science international, 2002. 130(2-3): p. 90-96.
14. Birincioğlu, İ., et al., Determination of skin wound age by using cytokines as potential markers. Journal of forensic and legal medicine, 2016. 44: p. 14-19.
15. Takamiya, M., et al., Wound age estimation by simultaneous detection of 9 cytokines in human dermal wounds with a multiplex bead-based immunoassay: an estimative method using outsourced examinations. Legal Medicine, 2009. 11(4): p. 186-190.
16. Ismail, S.A. and M.T. Hasan, Epidemiology of Road Traffic Accidents in Emergency Hospital in Erbil City. The Medical Journal of Tikrit, 2012. 18(182): p. 296-305.

Association between Genetic Polymorphism of 5-HTTLPR, and SGOT, SGPT and Catalase with Alcoholism of Iraqi People

Anwar A. Abdulla

Department of Biology, College of Sciences, University of Babylon, Iraq

Abstract

A case- control study on the relationship of 5-HTTLPR gene polymorphism with alcohol abuse among Iraqi individuals was conducted during period from December 2018 to June 2019. DNA was extracted from the blood samples of the study subjects. The genetic polymorphism analysis was conducted by Restriction Fragment Length Polymorphism (RFLP-PCR) for SLC6A4 gene. The results raveled that the odd ratio for the *LL* genotype was 1.697 indicating that homo mutant genotype were at higher risk for alcoholism than the wild type *SS*. This result showed that L allele frequency was (0.78) in alcoholism group and (0.53) in control group whereas S allele frequency was (0.22) in the alcoholism group and (0.47) in the control group. So, the results showed there was a significant difference in allele frequencies of *SLC6A4*gene between case and control ($P<0.01$; OR=1.697; 95% CI=0.86-1.64). There was a significant positive correlation between the SGOT activity and the genotype *of 5-HTTLPR* (*SS, SL, LL*).Additionally, there was a significantly increased level of liver enzymes (GOT and GPT) and catalase in serum samples of alcoholic males compared with non-alcoholic males.

Key Words: *SLC6A4, 5-HTTLPR, SGOT, SGPT, catalase.*

Introduction

Alcohol dependence and alcohol abuse are chronic disorders comprising a wide range of clinical symptoms. Given that drinking behaviors are jointly determined by genetic and environmental risk factors, alcohol consumption and alcohol use disorders are appropriate phenotypes for investigating gene- environment interactions [(1).] Alcohol consumption is a common, complex trait, and heavy alcohol use increases the risk of alcohol use disorders, and is recognized as, a problematic global problem threatening both individual development, family life and social life of a person [(2).] There is an evidence of a causal relationship between alcohol and at least 200 diseases including gastritis, pancreatitis, cardiovascular disease, liver cirrhosis, hepato cellular carcinoma, and gastric cancer [(3).] Ethanol is not stored in the body after ingestion because it is fully ingested oxidized during metabolization in the liver [(4).] The rate of ethanol metabolism determines the concentration of ethanol and its metabolite acetaldehyde in the different tissues, which in turn influences the effects of ethanol consumption on liver and the other organs. Serotonin is a signaling molecule with a widespread effect in the CNS, and has a very important role in different aspects of mammalian life, like food intake, emotion, mood, respiration, pain sensitivity, cardiovascular regulation, sexual behavior, learning and memory, circadian rhythm, sensorimotor activity, and cognition [(5).]

The human serotonin transporter (SETR) is a monoamine transporter protein, encoded by a single gene (SLC6A4, solute carrier family 6, member 4) located on the long arm of chromosome 17 (17q11.2). The serotonin transporter (5-HTT) is an important protein responsible for the active transport of serotonin into neurons, enterochromaffin cells and platelets [(6).] Twin studies have shown alcohol dependence (AD) to have a heritability of ~50–60% [(7, 8).] Among the genetic components, many of the genes that may contribute to the risk of alcohol phenotypes encode components of the dopamine, serotonin (5-HT), and acetylcholine neurotransmitter systems.

Material and Method

Study Subjects

A case- control study on the relationship of 5-HTTLPR polymorphism with alcohol abuse among Iraqi individuals was conducted during period from December 2018 to June 2019. Fifty blood samples of alcoholic male Iraqi people (with a history of alcohol abuse for more than seven years) were collected from the Institute of forensic medicine, Al-Yarmouk Teaching Hospital, Shaikh Zayed Hospital, and Ibn-Rushed Teaching Hospital/Baghdad, Iraq. The study subjects were men of ages mean of 35.04 years ± 10.89 SD. Additionally, 50 samples were collected from non-alcoholic subjects, as control group, with mean age of 34.30 years ± 10.86.

Blood Sampling

Five milliliters of blood were collected by vein puncture, two ml was put into EDTA tubes for molecular analysis and three ml put in separating gel tube, then was allowed to clot at room temperature for 30 minutes and then centrifuged at 2000 rpm/ 15 minutes. The sera were collected and stored at -20ºC until analysis.

Enzymatic Assay

The liver enzymes (GOT and GPT) and catalase in serum samples of alcoholic and non-alcoholic males were measured by Reflontron/ Germany in this study.

Genotyping of SLC6A4 Polymorphism

Preparation of Genomic DNA

The DNA from genome was prepared from blood samples (gSYNC™ DNA Extraction Kit) according to the instructions of the manufactured. Concentrations and purity of DNA were measured by Nano drop spectrophotometer (Apel/Germany).

PCR condition and Restriction Fragment Length Polymorphism (RFLP)

Two primers were selected (F-5- GGC GTT GCC GCT CTG AAT GC -3) and (R-5- GAG GGA CTG AGC TGG ACA ACC AC -3 ′) [9] to amplify fragments of (469 &512) bp for the detection of alleles. The specific designed primers were provided by AccuOligo/ Bioneer/ Korea. The PCR reaction was performed in a total volume 20 µl containg 10 p mole/µl of each primer, 1x Master mix(AccuPower® ProFiTaq PCR PreMix/ Bioneer/ Korea), and 0.15µg/µl genomic DNA. The reaction mixture was amplified in thermal cycler (Cleaver Scientific, UK). Initial denaturation was carried out at 95°C for 5 min and the target DNA was amplified in 40 cycles. Subsequently, each cycle consisted of denaturation at 95°C for 30 sec; followed by annealing at 63°C for 30 sec. Elongation was carried out at 72°C for 1 min. The final extension step was performed at 72°C for 10 min. Aliquots of products of amplified DNA were treated with *MspI* (restriction enzyme, SibEnzyme/ Russia). The PCR product (*5-HTTLPR)* was digested with restriction endonucleases in a total volume of 20µl containing 10 units of enzyme with buffers supplied by the manufacturer's instructions. The amplified PCR products were checked for the expected size on 2% (w/v) agarose gel and visualized after staining with ethidium bromide under ultraviolet. A 100bp DNA molecular weight marker (BioNeer/Korea) was used to measure the weight of the fragments [10].

Biostatistical consideration

The [11] program was used to analyze the difference factors in study variables. Chi-square test was used to compare differences among percentage at P-values of 0.05 and 0.01 probability. Odd ratio and 95% CI were estimated.

Results and Discussion

The study subjects were men of ages mean of 35.04 years ± 10.89 SD. Additionally, 50 samples were collected from non-alcoholic subjects, as control group, with mean age of 34.30 years ± 10.86, table (1).

Table (1): Distribution of ages of alcoholic male among cases and control

Age group (year)	Group	
	Cases	Control
< 30	16 (32.00%)	14 (28.00%)
30-40	20 (40.00%)	25 (50.00%)
> 40	14 (28.00%)	11 (22.00%)
Total	50	50

Results listed in table (1) indicated that the most common age group of cases(alcohol consumption) was

of 30-40 years (40.00%) followed by the group less than 30 years (32.00%), and more than 40 years (28.00%).

Blood Chemistry Analysis

The results of testing for GPT and GOT in serum from the cases as compared to the controls are presented in figure (1: A, B).

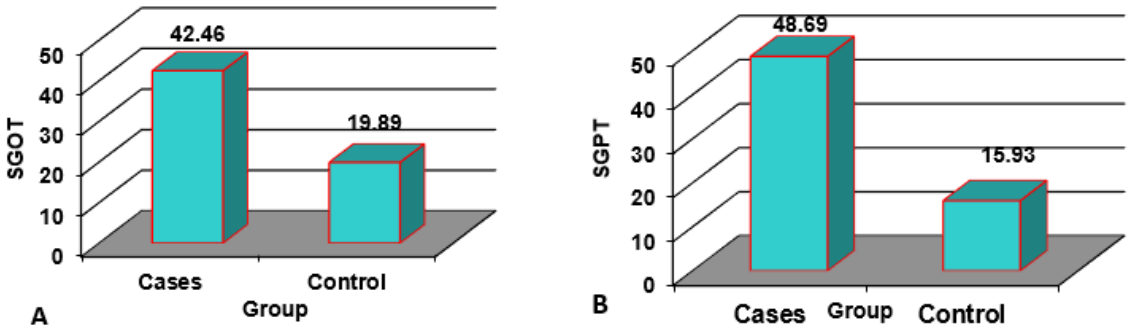


Figure 1: Comparison of liver enzymes; A (GOT) and B (GPT) in serum samples of alcoholic and non-alcoholic males

The results in the present study indicated that parameters measured (GPT and GOT), which are markers liver functions revealed significant differences between cases and controls, (42.46 ± 23.39 vs. 19.89 ± 11.31) (48.69 ± 21.31 vs. 15.93 ± 9.27) at (P<0.01) for GPT and GOT, respectively. Measurement of SGOT and SGPT becomes very important because these liver enzymes are the most important liver enzymes to represent groups or transaminase aminotransferase enzyme, which catalyze the keto acids into amino acids by transfer of amino groups. Serum enzymes are the most commonly used and sensitive biochemical markers for the assessment of liver disease. [12], who reported that alcohol is a toxin that is harmful to the liver and alcoholic liver disease and it is one of the leading causes of alcohol-related death. While [13], found consumption of alcohol causes several pathological changes in the liver. In respect to catalase there was significant difference between case and control group (363.42 ± 214.61 vs. 101.51 ± 51.64) at (P<0.01) respectively as show in figure 2.

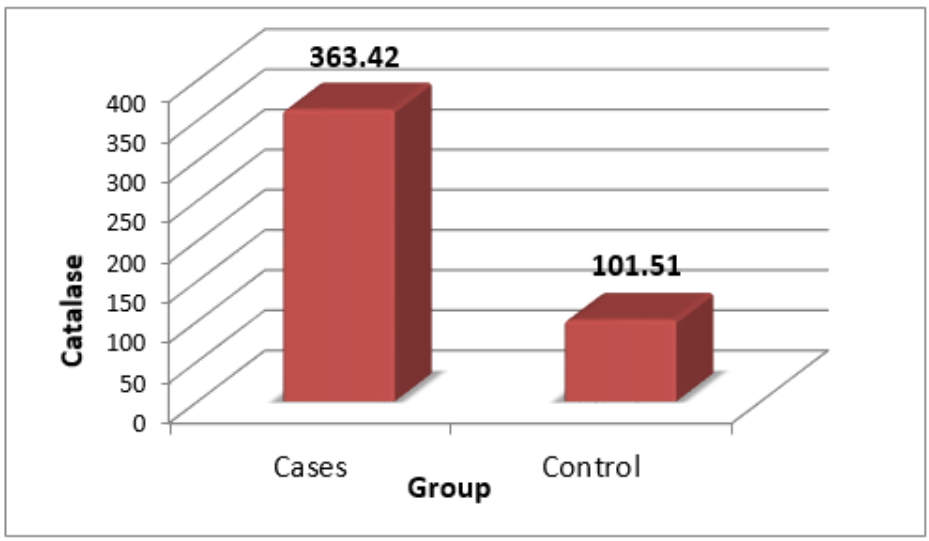


Figure 2: Comparison of catalase in serum samples of alcoholic and non-alcoholic males

The enzyme catalase has also been shown to oxidize ethanol into acetaldehyde within the peroxisomes. This process is hydrogen peroxide dependent. However, under normal physiological conditions, catalase plays only a minor role in ethanol metabolism,[14] but its contribution might be enhanced in the presence of higher amounts of hydrogen peroxide. Furthermore, catalase may be an alternative metabolic pathway for ethanol oxidation within the brain, where ADH and CYP2E1 appear to be of minor importance for ethanol metabolism [15].

[16] reported that the catalase activity increased in lower concentration of alcohol exposure, but in higher concentration of alcohol exposure catalase activity decreased compared to control groups . This finding suggested that catalase activity in the liver is changeable [17].

80-90% of alcohol breakdown in the liver results in the formation of acetaldehyde whose further metabolism in the cells leads to reactive oxygen species production (ROS) (18).

Acetaldehyde itself is a mutagenic and carcinogenic by product. It binds with DNA and interferes with DNA synthesis and repair mechanism. Furthermore, it results in tumor development [19].

Distributions of Genotypes and Allele Frequency of the 5-HTTLPR Polymorphisms in the *SLC6A4* Gene

The distribution of the observed *SLC6A4* gene genotypes and alleles frequencies in the control and cases individuals are shown in table (2).The highest genotype in the case group was homozygous *LL*(74.00%), while(18.00%) for homozygous *SS* genotype, and *LS* (8.00%).

Table (2): Distribution of genotype and allele frequency of 5-HTTLPR gene in cases and control.

Genotype of **5-HTTLPR**	**Cases No. (%)**	**Control No. (%)**	**Sig.**	**O.R. (95% C.I.)**
SS	9 (18.00%)	5 (10.00%)	0.0438 *	0.662 (0.78-1.56)
LS	4 (8.00%)	37 (74.00%)	0.0001 **	1.750 (0.86-1.71)
LL	37 (74.00%)	8 (16.00%)	0.0001 **	1.697 (0.86-1.64)
Total No.	50	50	---	---
Allele	Frequency	Frequency		
S	0.22	0.47	---	---
L	0.78	0.53	---	---
* (P<0.05), ** (P<0.01).				

The results from table (2) show significant differences in the frequencies of *SLC6A4* gene (LL) in the control and case groups (at P-value 0.01). The results raveled that the odd ratio for the LL genotype was 1.697 indicating that homo mutant genotype were a higher risk of alcoholism than the wild type SS. This result showed that L

allele frequency was (0.78) in alcoholism group and (0.53) in control group whereas S allele frequency was (0.22) in the alcoholism group and (0.47) in the control group as shown in table (2). So, the results showed there was a significant difference in allele frequencies of *SLC6A4*gene between case and control (P<0.01; OR=1.697; 95% CI=0.86-1.64).

Alleles of the 5-HTTPLR promoter have either a short (S) or long (L) copy of an imperfect repeat. The short or 'S' allele with 14 repeats was shown to have lower transcriptional activity than the long or 'L' allele with 16 repeats [20, 21]. [22] reported that the 'L' allele is associated with a predisposition to lower level of response to alcohol, which is in turn associated with the onset of alcoholism. While several previous studies that suggested an association of the S allele with alcohol and drug dependence [23, 24, 25]. [26] found out that there is an association between the 5-HTTLPR 'L' allele and the increased serotonin and platelet uptake pharmacologically; the serotonin transporter spans the plasma membrane 12 times.

Association between genotype of 5-HTTLPR and parameters

The association between 5-HTTLPR and SGOT, SGPT and catalase were investigated. The current study shows that the presence of an SGOT was significantly associated with genotype of 5-HTTLPR (*SS, SL, LL*) among cases at ($p < 0.05$), while SGPT was significantly among control at ($p < 0.05$). Regarding the association between genotype of 5-HTTLPR and catalase, there were no differences among case and control groups, table (3).

Table (3): Relationship between genotype of 5-HTTLPR and listed enzymes in cases and control.

Group	Genotype of 5-HTTLPR	Mean ± SD		
		SGOT	**SGPT**	**Catalase**
case	SS	29.16 ± 15.56 **b**	55.04 ± 15.05	389.82 ± 263.95
	SL	40.23 ± 19.95 **b**	47.84 ± 21.29	379.37 ± 243.08
	LL	61.06 ± 32.28 **a**	48.67 ± 26.12	273.13 ± 226.82
	LSD value	**20.77 ***	21.71 NS	186.97 NS
Control	SS	18.37 ± 11.25	19.42 ± 9.62 **a**	85.97 ± 24.13
	SL	20.50 ± 15.58	9.35 ± 2.15 **b**	111.22 ± 59.37
	LL	20.20 ± 11.18	15.80 ± 9.38 **ab**	104.24 ± 55.93
	LSD value	11.96 NS	**8.67 ***	55.92 NS
*** (P<0.05), NS: Non-Significant. Means having with the different letters in same column differed significantly**				

The results in the present study indicated that genetic polymorphisms of the 5-HTTLPR and SGOP in humans are linked to alcohol consumption and the incident of alcohol abuse.

Regarding the association between genotype of 5-HTTLPR and catalase, may be, duration of ethanol exposure and genetic background appear to be important variables in considering whether or not catalase changes as a response to ethanol.

Several studies have examined whether the *5-HTTLPR* polymorphism (L and S variants, LL, LS and SS genotypes) interacts with environmental risk factors to predict drinking outcomes [27, 28].

A repeat length polymorphism (5-HTTLPR) in the promoter of this gene has been shown to affect the rate of serotonin uptake and may play a role in drug dependence and other chronic neurological diseases [20, 21].

Some studies have suggested a possible involvement of the 5-HTTLPR genotype with alcoholism [22], smoking [29], suicidal behavior [30], and depression [31].

Conclusion

Researchers worldwide reported that there are

several underlying genetic factors that influence the development of alcoholism among individuals. However, to date there is only few published reports on this matter in alcoholism among Iraqi individuals. In this study there was a significant positive correlation between the SGOT activity and genotypes of 5-HTTLPR (*SS, SL, LL*). There was a significantly increased levels of liver enzymes (GOT and GPT) and catalase in serum samples of alcoholic compared to non-alcoholic males. The results in the present study indicated that genetic polymorphisms of the 5-HTTLPR and SGOP in humans are linked to alcohol consumption and conditions of alcohol abuse.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

References

1. Heath AC, Nelson EC. Effects of the interaction between genotype and environment. Research into the genetic epidemiology of alcohol dependence. Alcohol Research and Health. 2002. 26:193–201.
2. Dawson DA, The link between family history and early onset alcoholism: earlier initiation of drinking or more rapid development of dependence? J Stud Alcohol. 2000. 61(5):637–646.
3. World Health Organization (WHO). Global status report on alcohol and health. Geneva: World Health Organization. 2014 .
4. Das SK, Nayak PP, and Vasudevan DM. Consequences of ethanol consumption. J. Ind. Soc. Toxicol. 2005. 1: 1-10.
5. Anderson BM, Schnetz-Boutaud NC, Bartlett J, Wotawa AM, Wright HH, *et al*. Examination of association of genes in the serotonin system to autism. Neurogenetics. 2009. 10(3):209-16
6. Lesch KP, Gutknechtl S. Pharmacogenetics of the serotonin transporter. Prog Neuropsychopharmacol Biol Psychiatry. 2005. 29(6):1062-73.
7. Enoch MA, Goldman D. The genetics of alcoholism and alcohol abuse. Curr Psychiatry Rep 2001.3:144–51.
8. Ducci F, Goldman D. The genetic basis of addictive disorders. Psychiatr Clin North Am 2012.35:495–519.
9. Wendland JR, Martin BJ, Kruse MR, Lesch KP, Murphy, DL. Simultaneous genotyping of four functional loci of human SLC6A4, with a reappraisal of 5-HTTLPR and rs25531. Molecular Psychiatry. 2006. 11: 224–226.
10. Sambrook J, Russell D. Molecular cloning: A laboratory Manual 3rd Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. 2001.
11. SAS. Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA. 2012**.**
12. Okonkwo P, Oluchukwu B E, Raphael J O. Enzymes as markers of liver damage in apparently healthy alcohol drinkers resident in Vom community, International Journal of Biosciences (IJB). 2012. 2 (4), 90-95.
13. Rehm J, Samokhvalov AV, Shield KD. Global burden of alcoholic liver diseases, Journal of Hepatol. 2013. 59, (100)160–168.
14. Lieber CS. Microsomal ethanol-oxidizing system: the first 30 years (1968–1998)—a review. 1999. *Alcohol Clin Exp Res* ; **23**: 991–1007.
15. Zimatkin SM, Deitrich R A. Ethanol metabolism in the brain. *Addict Biol* . 1997. 2: 387–399.
16. Das S K , Vasudevan DM. Effect of ethanol on liver antioxidant defense systems: Adose dependent study. Indian Journal of Clinical Biochemistry. 2005. 20 (1) 80-84
17. Husain K, Somani SM. Interaction of exercise training and chronic ethanol ingestion on hepatic and plasma antioxidant system in rat. 1997. J.Appl. Toxicol. 17(3), 189-194.
18. Kim YH, Shin MJ. Effect of high taurocholate load on activities of hepatic alcohol metabolizing enzymes. Exp Mol Med. 2002. 34, 123-30.
19. Iarc A. Alcoholic beverage consumption and ethyl carbamate (urethane). IARC monographs on the evaluation of carcinogenic risks to humans. 2010. vol. 96, International Agency for Research on Cancer, Lyon.
20. Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Muller CR, Hamer DH, Murphy DL. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science. 1996.274:1527–

1531.

21. Lesch KP. Molecular biology, pharmacology, and genetics of the serotonin transporter: psychobiological and clinical implications In: Baumgarten HG, Gothert M (eds) Serotonergic Neurons and 5-HT Receptors in the CNS. Berlin, Germany: Springer. 1999.671–705.
22. Bleich S, Bönsch D, Rauh J, Bayerlein K, Fiszer R Frieling H. *et al*. Association of the long allele of the 5-HTTLPR polymorphism with compulsive craving in alcohol dependence. Alcohol Alcohol. 2007.42:509-512.
23. Sander T, Harms H, Podschus J, Finckh U, Nickel B, Rolfs A, Rommelspacher H, Schmidt LG. Allelic association of a dopamine transporter gene polymorphism in alcohol dependence with withdrawal seizures or delirium. Biol Psychiatry. 1997. 41:299–304.
24. Hallikainen T, Saito T, Lachman HM, Volavka J, Pohjalainen T, Ryyna¨nen O.P, *et al*. Association between low activity serotonin transporter promoter genotype and early onset alcoholism with habitual impulsive violent behavior. Molecular Psychiatry. 1999.4: 385–388.
25. Gerra G, Garofano L, Santoro G, Bosari S, Pellegrini C. *et al*. Association between low-activity serotonin transporter genotype and heroin dependence: behavioral and personality correlates. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics. 2004. 126B: 37–42.
26. Stoltenberg SF, Twitchell GR, Hanna GL, Cook EH, Fitzgerald HE, Zucker RA. *et al*. Serotonin transporter promoter polymorphism, peripheral indexes of serotonin function and personality measures in families with alcoholism. American Journal of Human Genetics. 2002. 114: 230–234.
27. Nilsson KW, Sjöberg RL, Damberg M, Alm P, Ohrvik J, Leppert, J, Lindström L, Oreland L. Role of the serotonin transporter gene and family function in adolescent alcohol consumption. Alcoholism: Clinical and Experimental Research. 2005. 29:564–570.
28. Kaufman J, Yang B, Douglas-Palumberi H, Crouse-Artus M, Lipschitz D, Krystal J.H, Gelernter J. Genetic and environmental predictors of early alcohol use. Biological Psychiatry. 2007. 61:1228–1234.
29. Gerra G, Garofano L, Zaimovic A, Moi G, Branchi B, Bussandri M. *et a*l.. Association of the serotonin transporter promoter polymorphism with smoking behavior among adolescents. Am J Med Genet B Neuropsychiatr Genet. 2005.135-B: 73-78.
30. Bellivier F, Szoke A, Henry C, Lacoste J, Bottos C, Nosten- Bertrand, M. *et al*. Possible association between serotonin transporter gene polymorphism and violent suicidal behavior in mood disorders. Biol Psychiatry. 2000.48:319-322.
31. Daniele A, Divella R, Paradiso A, Mattioli V, Romito F, Giotta F. et al.. Serotonin transporter polymorphism in Major Depressive Disorder (MDD), psychiatric disorders, and in MDD in response to stressful life events: causes and treatment with antidepressant. *In Vivo*. 2011.25:895-901.

Sensitive Simultaneous Estimation of Atorvastatin. Ca in Pure and Dosage Forms Via Developed CFIA Using 1,2 Naphthoquinone-4-Sulfonate as a Suitable Organic Agent

Bushra B. Qassim[1], Asmaa A. Zydan[2]

[1]Department of Chemistry, College of Science, University of Baghdad, Iraq

Abstract

Objectives: A sensitive visible spectrophotometric method and FIA/merging zones technique was developed for the determination of atorvastatin calcium in pure material and tablet dosage form.

Method: Atorvastatin calcium has a free carboxylic moiety in its structure, which when being deprotonated in basic medium facilitates associated the reagent with the drug. This method was based on the formation of red colored chromogen of drug with 1,2-Naphthaquinone-4-sulfonate(NQS) in basic medium (NaOH). The absorbance of the chromogens was measured at their respective wavelengths of maximum absorbance against the corresponding reagent blank

Results: The red colored product is directly completed in basic medium and exhibits maximum absorption at 525 nm. Different factors affecting the formation of the product and optimized in order to obtain the best conditions for the experiment and its stability were studied. Method validation was done over a concentration range of 2-10 and 1-20 µg/mL for batch and FIA method respectively.

Keywords: *Atorvastatin calcium; 1,2-Naphthoquinone-4-sulfonate, sodium hydroxide; Pharmaceutical formulation; CFIA/merging zones technique.*

Introduction

ATRV.Ca {[R-(R, R*)]-2-(4-flurophenyl)-β,δ-dihydroxy- 5(1-methylethyl)-3-phenyl-4-[phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1)} is the most commonly occurring drug in commercially available pharmaceutical formulations used for the clinical treatment of hypercholesterolemia [(1)]. Several methods have been described for the determination of ATRV.Ca HPTLC [(2)], (HPLC) in different pharmaceutical preparations, either alone [(3-8)] or with other active ingredients [(9-17)], electrochemical [(18,19)], spectrofluorimetric [(20)] and capillary electrophoresis [(21)] methods have been developed for the analysis of ATRV.Ca in pharmaceutical preparations. Various spectrophotometric methods have been reported for the determination of ATRV [(9,15,22-26)] from its individual and combined formulations with other active ingredients. The official procedures in pharmaceutical preparations utilize non-aqueous titration method [(27)]. Kinetic methods have certain advantages in pharmaceutical analysis regarding selectivity and elimination of additive interferences, which affect direct spectrophotometric methods. Some specific advantages that the spectrophotometric FIA methods possess are as follows [(28)].

- High selectivity since they involve the measurement of the absorbance as a function of reaction time instead of measuring the concrete absorbance value.

- Simple and fast methods because some experimental steps such as filtration, extraction, etc.

-Other active compounds present in the commercial dosage forms may not interfere if they are resisting the chemical reaction conditions established for the proposed method.

- Colored and/or turbid sample background may possibly not interfere with the determination process.

Corresponding author:
Asmaa A. Zydan
asmaahmed930@yahoo.com

Materials and reagent

A standard solution of ATRV.Ca ($C_{66}H_{68}CaF_2N_4O_{10}$= 1155.34 g mol^{-1}, Sigma Aldrich). A 0.05 g of pure ATOR was dissolved in 100 mL methanol to prepare 500 µg/mL of standard ATRV.Ca. A standard stock solution of NQS ($C_6H_4COCOCH:CSO_3$ Na = 260.20 g mol^{-1}, Fluke) A 0.05 M of Reagent was prepared by weighing a 1.3 g of reagent and dissolving in distilled water and made up to 100 mL with it. A stock solution of NaOH (40 g mol^{-1}, BDH) A NaOH 1M was prepared by weighing a 4g of oxidant and dissolving in distilled water and made up to 100 mL with it.

Instrumentation

A Optima, Photomech 301-D^+, UV-Visible Spectrophotometer single beam recording spectrophotometer (Japan) was used for performed all absorbance and spectral measurements of FIA procedures, for the absorbance measurements as peak height through Kompensograph C1032, Siemens or absorbance with digital multimeter (DT9205A, China). Inside the detection unit, there is a flow cell (quartz silica (QS), 1 cm) with 80 µL internal volume. A Shimadzu UV-1800 (Japan) double-beam spectrophotometer were used for batch procedure, and quartz cuvette with an optical path length of 1 cm. A one channel manifold was employed for the FIA/merging zones system. A peristaltic pump of four channels (Shenchen, LabM1) used for pumping the distilled water as a carrier stream of through the valve (homemade, six-three injection valve (merging zone version)), which moves at 90° and three Teflon loops were loaded with the sample solutions and reagent. Mixing coil that was manufactured from glass with 2 mm (I.D). A single channel manifold system in FIA was shown in Figure.1.

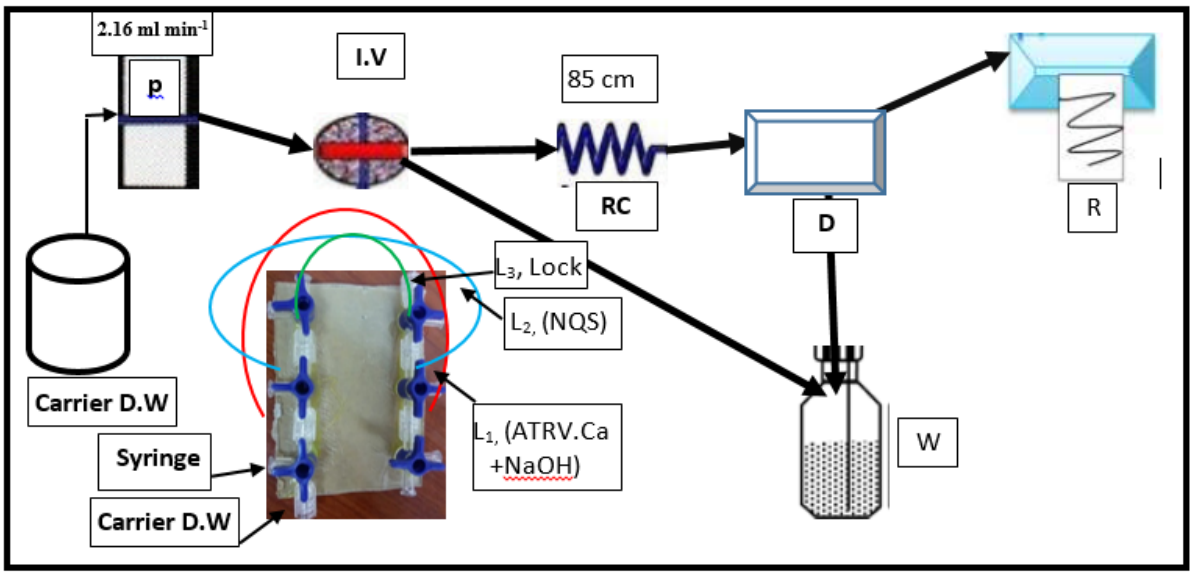


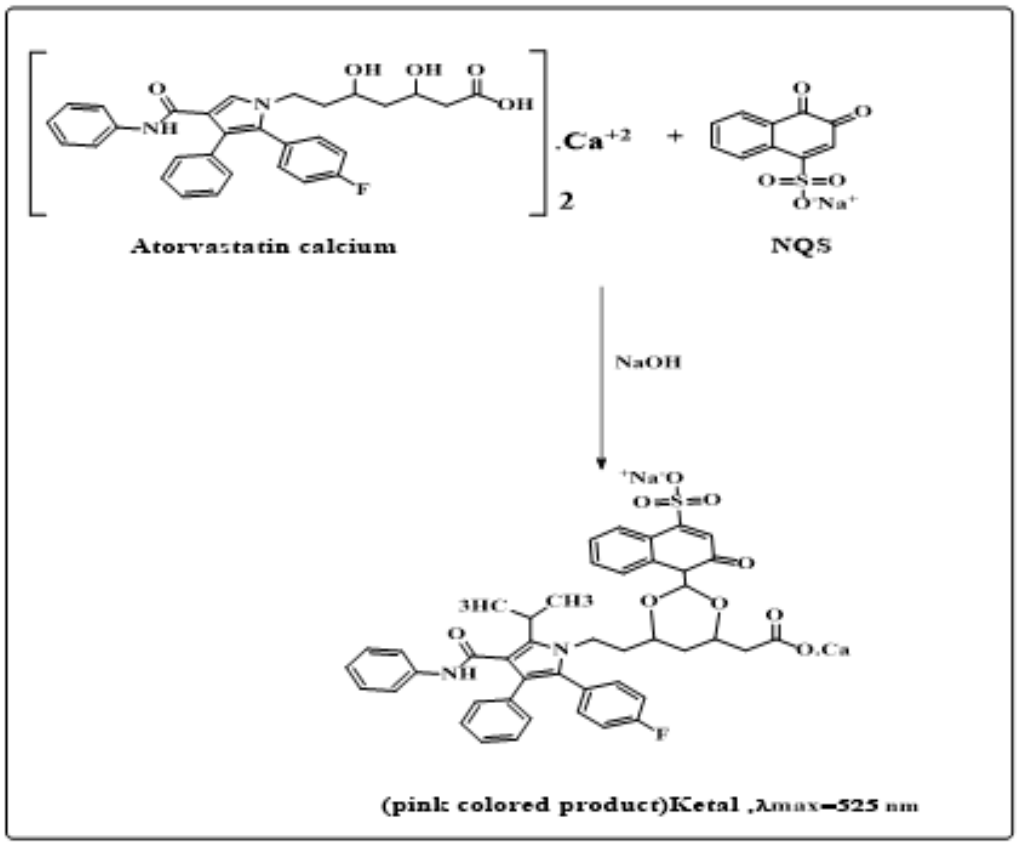
Figure.1. Manifold employed for FIA-Spectrophotometric determination of ATRV.Ca, where: I.V, injection valve; R.C, reaction coil; P, peristaltic pump; D, detector; R, Recorder; W, waste.

Assay procedure for tablets

The solutions of pharmaceutical preparations by appropriate amount equivalent 0.02 g of the each sample was weighting that be equal to 200 µg mL^{-1} of resulting powder were dissolved in 100 ml volumetric flask with 25 mL of methanol for and then shaken and filtered into a volumetric flask of 100 mL. The residue was washed and diluted to volume with distilled water to gain 200 µg/mL of statin drugs.

Mechanism of the Reaction

The suggested mechanism of this reaction of ATRV. Ca with (NQS) in basic medium to form a red complex directly as shown in scheme (I). The stoichiometry of the reaction between ATRV.Ca and NQS was investigated [22].



Scheme I: The suggested mechanism of the reaction between ATRV.Ca with (NQS) complex

Result and Discussion

Batch spectrophotometric determination: In the subsequent experiments, 4 µg mL^{-1} of ATRV.Ca was taken in 10 mL final volume and performed by changed one factors at a time and keeping the other parameters fixed and observing the effects of the product on the absorbance.

Concentration of NQS:

The effect of various concentration of NQS was investigated using different concentration ranging from (0.001-0.01 M). A concentration of 0.005 M reagent gave the highest absorbance and was chosen for further experiments.

Concentration of sodium hydroxide: The effect of concentration of sodium hydroxide was investigated by carrying out the reaction using different volumes of NaOH ranging (0.005-0.2 M). The maximum absorbance was obtained upon 0.05 M.

Calibration curve of classical method:

The impact of using different concentration of ATRV.Ca (1,2, 2.3, 2.5, 3, 4, 5, 6, 7, 8,10,12) µg mL^{-1} were examined with stabilized the other parameters. Transfer set of volumetric (10 ml) contain 2.5 mL of (NQS) (0.02 M) followed by 1 mL of NaOH (0.5 M) then an increasing volumes from standard solutions (100 $\mu g.ml^{-1}$). The solutions had been diluted to the marked using distilled water. The reaction mixture measured the maximum absorption of the colored product at 525 nm. The standard curve was constructed and linear range (2-8) $\mu g.mL^{-1}$ for the determination of ATRV.Ca, as shown in Figure (2).

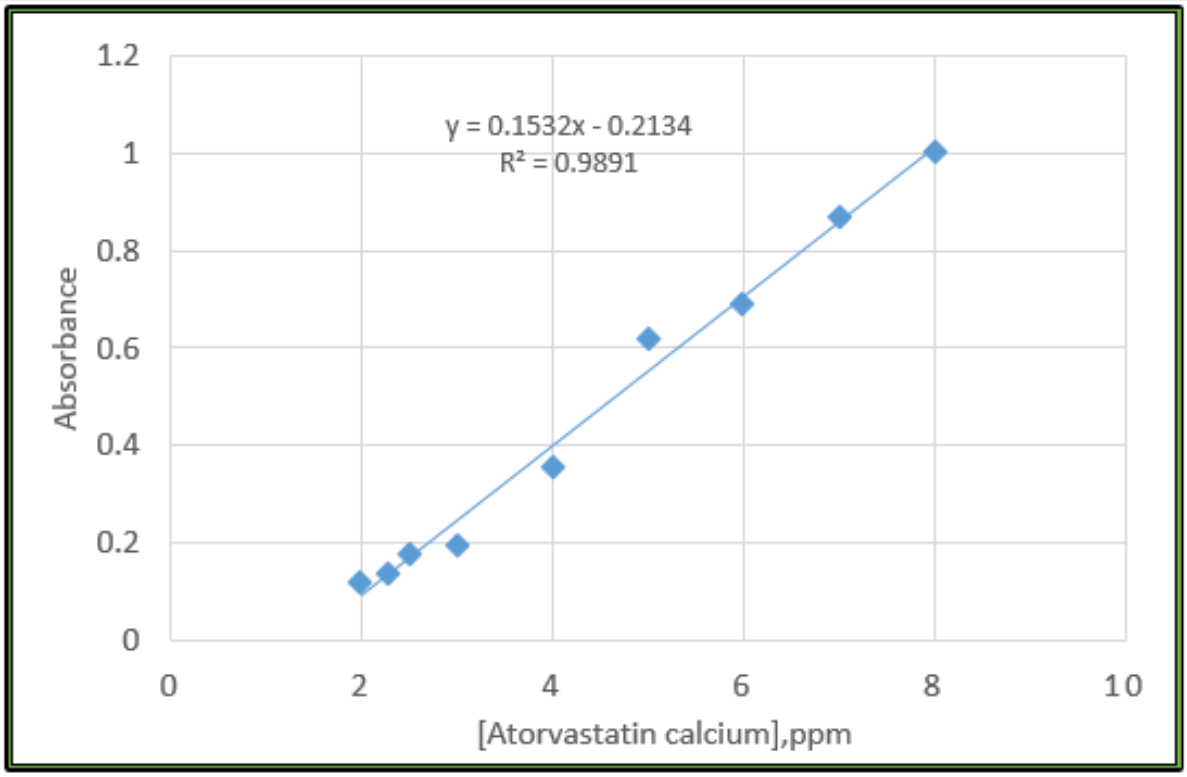


Figure.2. Calibration curve of reaction between ATRV.Ca and NQS in basic medium

Calculations of stability constant:

$K = 1-\alpha/\alpha^3C^2$ …….. (1), (α) (degree of dissociation) can be written as follows:

α = Am - As /Am…….. (2), Am; As are the values of absorbance of the aqueous solution including a more than enough and stoichiometric amount of the reagent.

Optimization of the FIA system conditions

Initial studies were directed towards the optimization of the experimental conditions for FIA system.

Effect of reagent and basic medium: Optimum concentration of the reagent was studied by injecting different concentrations (0.005-0.08) M using IV. The results indicated that the 0.05 M gave the good repeatability with highest value of absorbance.

NaOH found to be a useful basic medium for this reaction, different concentrations of NaOH were also studied in the range of 0.01 to 0.08 M. The result referred to increase the value of absorbance with increasing the concentrations of basic medium up to 0.02 M and after this concentration the value of absorbance decreased. As a result, 0.02 M was chosen for the subsequent experiments.

Effect of physical parameters

Effect of optimum total flow rate

Optimum flow rate was studied using a range changed flow rates (1.2-2.6) $mLmin^{-1}$. The result demonstrates that a flow rate of 2.16 $mLmin^{-1}$ gave the highest absorbance value.

Effect reaction coil length and injection volume

Optimum length of reaction coil was studied in range of 85-250 cm. A best absorbance with acceptable repeatability was gained from the length of 85 cm. Absorbance decreased upon using a coil length of more than 85 cm.

Various volumes of injector loop were tested in this study. Effect of injected sample volume (L_1) was changed (58.875, 68.687, 88.312 and 127.562) µL and the volume of injection reagent (L_2) also studies was in deferent volume (68.687-127.562) µL. a 58.875, 68.687

µL for L_1, L_2 respectively was used in the next experiments.

Method validation

The linearity of the calibration graph for FIA method was obtained by injecting a series of solutions of ATRV. Ca (1-20 µg mL^{-1}) prepared from stock solution (100 µg mL^{-1}) with 0.02 M of basic medium as shown in figure (3). A portion of NQS (0.005 M) was injected as summarized in Table 1.These small points were referred to high reproducibility and repeatability of the developed FIA contrasted with the batch procedure.

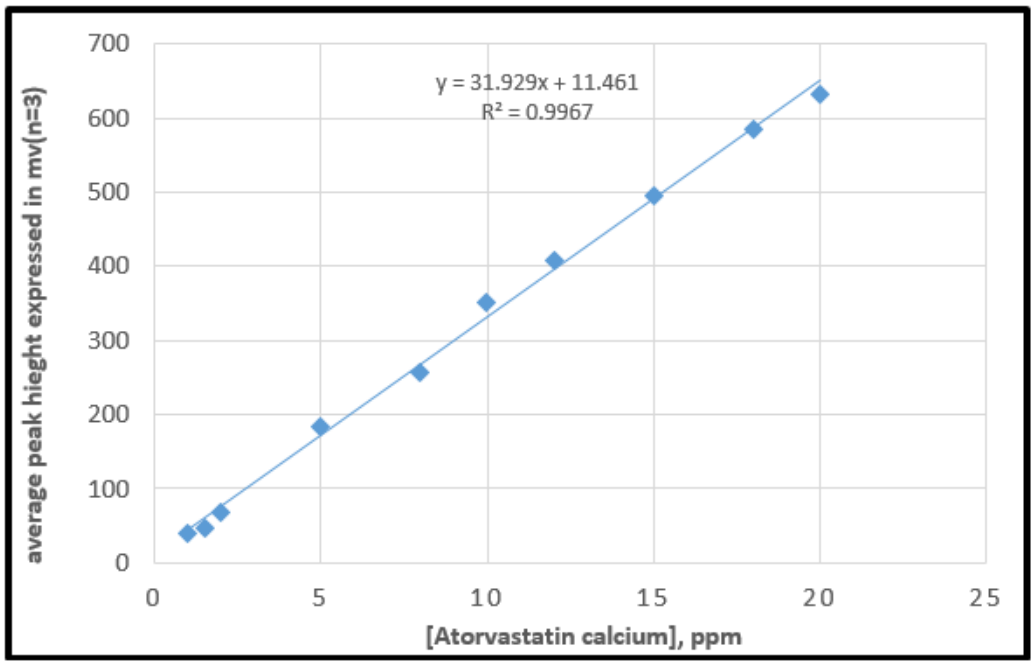


Figure .3. Linear calibration curve for determination of atorvastatin calcium with NQS using the developed FIA system.

Table 1. Summary of optical characteristics

Parameters	Batch method	FIA method
Linear range (µg mL-1)	2-8	1-20
Regression equation	y = 0.1532x + 0.2134	y = 31.929x +11.461
Correlation coefficient (r)/ r2	0.9945	0.9983
Linearity (r2 %)	98.91	99.67
Relative standard deviation (RSD %)	0.21 (at 5 ppm)	0.3 (at 10 ppm)
Slope (b); (mL.µg-1)	0.1532	31.929
Intercept (a); (a = y– b x)	0.2134	11.461
Standard deviation of intercept (Sa)	7.07 × 10-5	4.47 × 10-4
Confidence limit of intercept (a) = a ± tSa	0.2134 ± 0.0007	11.461± 0.163
Standard deviation of slope (Sb)	8.49 × 10-4	5.74 × 10-4
Confidence limit of slope (b) = b ± tSb	0.1532 ± 0.0002	31.929 ± 0.0049
Standard deviation of the residuals;	0.38 × 10-4	0.015
Average of recovery (%)	99.66	100.4
Limit of detection (LOD)	0.06	0.002
Limit of quantification (LOQ)	0.2	0.006
Sample through put (h-1)	10	68

Application of the proposed method using pharmaceutical:

The proposed batch and FIA method was successfully applied for estimation ATRV.Ca in tablets by the analysis of three types in two different concentrations of ATRV.Ca tablets and the results are listed in Table 2. In the direction of assessing the proficiency of the method. The statistical comparison between proposed and official methods using the student t- and F-test [27] indicated that the calculated values for F-test were (2.57) and (1.22), t-test values were (2.08) and (1.13) for the FIA and batch methods, respectively, were less than the theoretical one of F-test = 6.388 (n1 + n2 – 2 = 6) and t-test = 2.31.

Table 2. Application of the proposed batch and FIA and official methods for estimation of ATRV.Ca in tablets.

Dosage form	Proposed methods						Official method-od recovery (%)
	Batch			FIA-merging zones			
	Present conc. ($\mu g\ mL^{-1}$)	Rec (%)	RSD (%)	Present conc. ($\mu g\ mL^{-1}$)	Rec (%)	RSD (%)	
AVAS Tablets (10 mg/tablet) 3 100.30 0.41 MICRO LABS LIMITED 5 99.92 0.09				10 99.80 0.28 15 101.10 0.14			100.60
AVAS Tablets (20 mg /tablet) 3 101.30 0.20 MICRO LABS LIMITED 5 101.00 0.19				10 100.70 0.15 15 99.50 0.20			99.20
LIPODAR Tablets (10mg /tablet) 3 99.00 0.21 Dar Al Dawa, Na,ur - Jordan 5 99.40 0.18				10 98.20 0.22 15 100.13 0.10			100.50
LIPODAR Tablets (20mg /tablet) 3 99.67 0.52 Dar Al Dawa, Na,ur – Jordan 5 101.20 0.089				10 101.00 0.30 15 100.93 0.09			101.00
ATEROZ Tablets (20mg /tablet) 3 98.67 0.93 bilim 5 100.60 0.04				10 100.50 0.45 15 99.80 0.04			99.90

Conclusion

The developed methods were selective, rapid, simple and inexpensive and exhibits a fair degree of accuracy and precision . The method does not involve any critical reaction conditions. The proposed method can serve as an alternative method for the routine analysis of ATRV. Ca in pure drug and in pharmaceutical formulations. The methods is based on formation of a red condensation adduct upon reaction of ATRV.Ca and NQS in (NaOH). The method has low detection limit and high sample throughput. The proposed methods that followed Beer's law and give a good application for the pharmaceutical preparation. The wide applicability of the FIA method for daily quality control is well proven by analyzing the assay of ATRV.Ca at effect concentration level in dosage forms

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

References

1. Shitara Y, Sugiyama Y Pharmacokinetic and pharmacodynamics alterations of hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors: drug–drug interactions and interindividual differences in transporter and metabolic enzyme functions. Pharmacol Ther (2006) 112: 71-105.
2. Jamshid A, Nateghi AR HPTLC determination of atorvastatin in plasma. Chromatographia (2007) 65: 763-766.
3. Li WL, Zhong QY, Wen Q HPLC determination of content of atorvastatin calcium capsules and its related substances. Chinese Journal of Pharmaceutical Sciences. (2007); 12: 23-27
4. Nováková L, Šatínskýa D, Solicha P HPLC methods for the determination of simvastatin and atorvastatin. Tr AC Trends in Analytical Chemistry (2008) 27: 352-367.
5. Erturk S, Aktas ES, Ersoy L, Ficicioglu S An HPLC method for the determination of atorvastatin and its impurities in bulk drug and tablets. J Pharm Biomed Anal (2003) 33: 1017-1023.
6. Petkovska R, Cornett C, Dimitrovska A Development and validation of rapid resolution RP-HPLC method for simultaneous determination of atorvastatin and related compounds by use of chemometrics. Analytical Letters (2008) 41: 992-1009.
7. Altuntas TG, Erk N Liquid chromatographic determination of atorvastatin in bulk drug, tablets and human plasma. Journal of Liquid Chromatography & Related Technologies (2004) 27: 83-93.
8. Pasha MK, Muzeeb S, Basha SJ, Shashikumar D, Mullangi R, et al. Analysis of five HMG-CoA reductase inhibitors- atorvastatin, lovastatin, pravastatin, rosuvastatin and simvastatin: pharmacological, pharmacokinetic and analytical overview and development of a new method for use in pharmaceutical formulations analysis and in vitro metabolism studies. Biomed Chromatogr (2006) 20: 282-293.
9. Sonawane SS, Shirkhedkar AA, Fursule RA, Surana SJ Application of UV-spectrophotometry and RP-HPLC for simultaneous determination of atorvastatin calcium and ezetimibe in pharmaceutical dosage form. Eurasian Journal of Analytical Chemistry (2006) 1: 31-41.
10. Shah DA, Bhatt KK, Mehta RS, Shankar MB, Baldania SL, et al. Development and validation of a RP-HPLC method for determination of atorvastatin calcium and aspirin in a capsule dosage form. Indian Journal of Pharmaceutical Sciences (2007) 69: 546-549.
11. Mohammadi A, Rezanour N, Dogaheh MA, Bidkorbeh FG, Hashem M, et al. A stability-indicating high performance liquid chromatographic (HPLC) assay for the simultaneous determination of atorvastatin and amlodipine in commercial tablets. J Chromatogr B Analyt Technol Biomed Life Sci (2007) 846: 215-221.
12. Raja Rajeswari K, Sankar GG, Rao AL, Seshagirirao JVLN RP-HPLC method for the simultaneous determination of atorvastatin and amlodipine in tablet dosage form. Indian Journal of Pharmaceutical Sciences (2006) 68: 275-277.
13. Kumar Talluri MVN, Kalyankar A, Ragampeta S Synchronized separation of atorvastatin-an antihyperlipidemic drug with antihypertensive, antidiabetic, antithrombotic drugs by RP-LC for determination in combined formulations. Journal of Pharmaceutical Analysis (2012) 2: 285-292.
14. U Seshachalam U, Kothapally Chandrasekhar B HPLC analysis for simultaneous determination of atorvastatin and ezetimibe in pharmaceutical formulations. Journal of Liquid Chromatography & Related Technologies (2008) 31:714-721.
15. Nakarani NV, Bhatt KK, Patel RD, Bhatt HS Estimation of atorvastatin calcium and fenofibrate in tablets by derivative spectrophotometry and liquid chromatography. J AOAC Int (2007) 90: 700-705.
16. Chaudhari BG, Patel NM, Shah PB, Patel LJ, Patel VP Stability-indicating reversed-phase liquid chromatographic method for simultaneous determination of atorvastatin and ezetimibe from their combination drug products. J AOAC Int (2007) 90: 1539-1546.
17. Kadav AA, Vora DN Stability indicating UPLC method for simultaneous determination of atorvastatin, fenofibrate and their degradation products in tablets. J Pharm Biomed Anal (2008)

48: 120–126.

18. Erk N Development of electrochemical methods for determination of atorvastatin and analytical application to pharmaceutical products and spiked human plasma. Critical Reviews in Analytical Chemistry (2004) 34: 1-7.
19. Korany MA, Hewala II, Abdel-Hay KM Determination of etofibrate, fenofibrate, and atorvastatin in pharmaceutical preparations and plasma using differential pulse polarographic and square wave voltammetric techniques. J AOAC Int (2008) 91: 1051-1058.
20. Sharaf El-Din MMK, Salama FMM, Nassar MWI, Attia KAM, Kaddah MMY Validated spectrofluorimetric method for the determination of atorvastatin in pharmaceutical preparations. Journal of Pharmaceutical Analysis (2012) 2: 200-205.
21. Al-Shehri MM A validated capillary electrophoresis method for simultaneous determination of ezetimibe and atorvastatin in pharmaceutical formulations. Saudi Pharmaceutical Journal (2012) 20: 143-148.
22. Khan MR, Deepti J Simultaneous spectrophotometric determination of atorvastatin calcium and amlodipine besylate in tablets. Indian Journal of Pharmaceutical Sciences (2006) 68: 546-548.
23. Stanisz B, Rafa W Development and validation of UV derivative spectrophotometric method for determination of atorvastatin in tablets. Chemia Analityczna (2008) 53: 417-428.
24. Erk N Extractive spectrophotometric determination of atorvastatin in bulk and pharmaceutical formulations. Analytical Letters (2003) 36: 2699-2711.
25. Nagaraj, Vipul K, Rajshree M Simultaneous quantitative resolution of atorvastatin calcium and fenofibrate in pharmaceutical preparation by using derivative ratio spectrophotometry and chemometric calibrations. Anal Sci (2007) 23: 445-451.
26. Ashour S, Khateeb M A novel use of oxidative coupling reactions for determination of some statins (cholesterol-lowering drugs) in pharmaceutical formulations. Spectrochim Acta A Mol Biomol Spectrosc (2011) 78: 913-917.
27. The British Pharmacopoeia (2010) The Stationary Office, vol 2, London.
28. Bushra B. Qassim, Ahmed A. Alwan, New CFIA / Merging Zones Technique for Determination of Captopril in Pure and Pharmaceutics Dosage forms through the Oxidation/ Reduction Reaction of Drug with Cu(II)- Neocuproine Complex via Spectrophotometric Detection, International Journal of Science and Research (IJSR), 2017, DOI: 10.21275/ART20177121.
29. Chatfield C., 1975, ‘’Statistics of Technology ‘’. Chapman and Hall.

Gall Bladder Wall Thickness: Sonographic Accuracy and Laparoscopic Cholecystectomy Conversion Rate, Evaluated by Histopathology

Mohammad Faraj Dawod Al. Jafary [1] , Ammar Fouad Abdulrazaq[1] , Ayman Nadeem Kadum[3]

[1]Surgeon/ M.B.Ch.B, F.I.C.M.S (GS), F.I.C.M.S (GIT)/ Department of Surgery, Al-Yarmouk Teaching Hospital, Baghdad, Iraq

Abstract

Background: Preoperative prediction of a difficult laparoscopic cholecystectomy can help the patient as well as the surgeon to prepare better for intraoperative risk and the risk of conversion to open Cholecystectomy.

Aim of study: Evaluation the impact of gall bladder wall thickness, on the outcome of laparoscopic cholecystectomy and conversion rate to open cholecystectomy assessed by sonography preoperative and postoperative measurement of gall bladder wall thickness by histopathology.

Patients and Methods: A prospective study conducted in the surgical unit, Department of surgery, Baghdad Teaching Hospital between November 2010 and November 2011. Abdominal Sonography performed in 110 consecutive patients before laparoscopic cholecystectomy. The surgeon re-verified sonographic finding in operative room, and postoperatively, the gall bladder specimens were sent for histopathological measurement of wall thickness.

Results: Out of 110 patients with cholecystolithiasis on sonography, we encountered easy laparoscopic cholecystectomy in 80 patients (72.7%), difficult laparoscopic cholecystectomy in 24 (21.8%) and the procedure was converted to open cholecystectomy in six patients (5.5%). The difference between Sonographic and histological measurement was within 1 mm in 102 patients (92.7%), and the other 8 patients was with 1.5 mm (7.3%) with sensitivity of (100%), specificity of (83.3%) and accuracy of (97%).

Conclusion: An accurate preoperative diagnostic sonography is mandatory for planned laparoscopic gall bladder surgery to provide information for the selection of the most appropriate approach and avoid intraoperative difficulties and surprises. On sonography gall bladder wall thickening is the most sensitive indicator of technical difficulties during laparoscopic cholecystectomy. Such difficulties may require conversion to laparotomy.

Keywords: *Laparoscopic cholecystectomy, gall bladder wall thickness, sonography, Iraq.*

Introduction

Cholelithiasis has a high prevalence. Although cholelithiasis only becomes symptomatic in about 50% of patients, cholecystectomy is a common surgical procedure [(1)]. Gallstones are one of the major causes of morbidity in Western society. Prevalence of people with gallstones, whether symptomatic or asymptomatic, varies from 5 to 22% [(2)]. In Iraq, operations of gallbladder (GB) represent a considerable fraction of total operations conducted in hospitals. This indicates that the disease is relatively important in Iraq [(3)]. Recently, laparoscopic cholecystectomy (LC) has become the gold standard for treatment of symptomatic gallstones, due to lower morbidity, shorter hospital stay, earlier return to regular daily activities, less postoperative pain and a significant reduction in the incidence of wound complications and postoperative ileus has been documented in patients undergoing LC [(4, 5)]. In addition to numerous advantages, also technical limitations of laparoscopy should be mentioned, which - in the presence of chronic inflammation resulting in

pericystic adhesions and conglutination – increase the risk of undesirable conversion from LC to open surgery [6]. The severity of acute inflammatory change influences the degree of surgical difficulty. GB wall thickening and pericholecystic fluid are indicators of inflammation in patients with acute cholecystitis [7]. The most common risk factors for conversion include a thickened GB wall, past acute cholecystitis, diabetes mellitus, past upper gastrointestinal tract surgeries, age > 65 years and male gender [8, 9]. The selection of the patient who will undergo LC is important, and the most frequently used method other than the clinical evaluation, is radiological examination (ultrasonography) [2]. Preoperative classification of patients into a high risk group would be an objective factor facilitating the surgeon's decision on possible conversion [9]. A preoperative GB ultrasound, which documents a thick GB wall (> or =3 mm) with calculi, is a clinical warning for a difficult LC which may require conversion to an open surgery. In a study, it was found that the rate of conversion was 60% in case of thickened GB wall while 12% in case of normal GB wall [10]. Variable results have been reported in the past about the sensitivity, specificity, positive predictive value, and accuracy of GB wall thickening as an indicator of surgical conversion [7].

The success of any laparoscopic operation depends on both proper patient selection, and the technical skill and experience of the laparoscopist [11]. The aim of the study was to evaluate the impact of GB wall thickness on the outcome of LC and the conversion rate to open cholecystectomy assessed by preoperative sonography and postoperative measurement of GB wall thickness by histopathology.

Patients and Methods

Study Design and Setting: This was a prospective study that was conducted in the surgical unit, Department of Surgery, Baghdad Teaching Hospital during a period of one year from Nov, 2010 – Nov, 2011.

Study Population and sample size: The study included patients with feature of chronic calculus cholecystitis who were prepared for LC, so the total number was 110. Patients who had previous abdominal surgery and features of acute cholecystitis (clinically and by investigation) were excluded.

Workup: All patients were evaluated by sonography after fasting at least six hours, the wall of GB was carefully evaluated and consider as thick when it is (> 3mm), size and capacity of GB, pericystic fluid collection and biliary system status was evaluated as well as number of GB stones also recorded. Hematological and biochemistry investigation were done. GB wall thickness was measured postoperatively by histopathology; grossly and microscopically as. Initial Procedure of histopathological examination done by.

Measurements:

- GB: length × maximum diameter (cm).
- Cystic duct: length × maximum diameter (cm).
- Lymph node: number and maximum diameter (cm).
- Open longitudinally from the fundus towards the cystic duct with blunt-ended scissors, draining off the bile and noting any contents.
- Photograph if appropriate.
- Paint the external serosal and adventitial aspects if there is any suspicion of tumor.
- Fixation by immersion in 10% formalin for 36 – 48 hours.

The difficulty of procedure was evaluated by: -

1. Clearity of calot's triangle (peritoneal adhesion) length and width of cystic duct.

2. Handling of GB during procedure and ability to perforate it.

3. Dissection of GB from its liver bed and bleeding from it.

4. Extraction of GB to outside.

All patients were undergoing surgery which was done by senior general surgery and resident using closed methods with four ports. Histopathological examination done by senior histopathology. LC considered easy when there is minimal adhesion involving the omentum, only attaches to the fundus and body of GB, and easily separated. Difficult LC when there is sever adhesion involving calot's triangle.

Statistical analysis: The data analyzed using Statistical Package for Social Sciences (SPSS) version 25. The data presented as mean, standard deviation

and ranges. Categorical data presented by frequencies and percentages. Chi–square test was used to assess statistical association between certain variables and GB Wall thickness. A level of p – value less than 0.05 was considered significant.

Results

In this study, mean age of patients was 42.7 ± 8.4 years; and 82.6% were females. By U/S, 64.5% of patients had GB with wall thickness ≤ 3 mm. We noticed that LC was easy in 72.7% of cases, difficult in 21.8%, and converted to open surgery in 5.5% of cases as shown in table (1).

Table 1: Distribution of study patients by certain characteristics

Variable	No. (n=110)	Percentage (%)
Age (Years)		
< 30	24	21.8
30 - 49	64	58.3
≥ 50	22	19.9
Gender		
Male	19	17.4
Female	91	82.6
GB Wall thickness (mm)		
≤ 3	71	64.5
> 3	39	35.5
Type of operation		
Easy LC	80	72.7
Difficult LC	24	21.8
Converted to open surgery	6	5.5

The difference between sonographic and histopathologic measurement was below 0.5 mm in 80 patients (72.5%) and it was between 0.5 and 0.99 mm in 22 patients (20%), so it was within 0 – 1 mm in 102 patients (92.7%), and in other 8 patients the difference was within 1.5 mm from GB wall thickness as shown in table (2)

Table 2: Difference of histopathological and ultrasound measurement of gall bladder wall thickness.

Difference between measurements (mm)	No. (n=110)	Percentage (%)
No difference	44	40.0
0.1 – 0.49	36	32.5
0.5 – 0.99	22	20.0
1 – 1.5	8	7.5

In table 3, 83.3% of cases who were converted to open surgery had GB wall thickness > 3 mm by U/S with a significant association (P= 0.001) between GB Wall thickness by U/S and type of operation.

Table 3: Association between GB Wall thickness by U/S and type of operation

Type of operation	GB Wall thickness by U/S (mm)		Total (%) n= 110	P - Value
	≤ 3 (%) n= 71	> 3 (%) n= 39		
Easy LC	64 (80.0)	16 (20.0)	80 (72.7)	0.001
Difficult LC	6 (25.0)	18 (75.0)	24 (21.8)	
Converted to open surgery	1 (16.7)	5 (83.3)	6 (5.5)	

In table 4, 90% of cases who complained from GB perforation had GB wall thickness > 3 mm by U/S with a significant association (P= 0.001) between GB Wall thickness by U/S and postoperative complication.

Table 4: Association between GB Wall thickness by U/S and postoperative complication

Postoperative complication	GB Wall thickness by U/S (mm)		Total (%) n= 110	P - Value
	≤ 3 (%) n= 71	> 3 (%) n= 39		
No	68 (72.3)	26 (27.7)	94 (85.5)	0.001
GB Perforation	1 (10.0)	9 (90.0)	10 (9.1)	
Bleeding	2 (33.3)	4 (66.7)	6 (5.5)	

Discussion

Since the Introduction in 1985, LC had been the procedure of choice in treatment of symptomatic gall stone [12]. But some of the planned LC needs conversion due to various factors, it would be useful in advance to know which one would require conversion, so that experienced laparoscopic surgeon could be scheduled to minimize conversion rate. And since the 1970s, ultrasound has become known as a quick, non-invasive and reliable tool to diagnose GB disease [13, 14]. Ultrasound is very sensitive for the diagnosis of gall stones, but few data are available to assess its diagnostic value for the GB wall thickness [15]. We assessed the value of sonography for patients with gall stone disease prior to LC. This study corroborates the well-established high accuracy (97%) of sonography for assessing the thickness of GB wall thickness.

In this study, we found that increase GB wall thickness on preoperative ultrasound which encountered in 39 patients out of 110 patients (35.5%) were associated with increase operative difficulty in 18 patients out of 39 patients, and our conversion rate to open surgery in six patients out of 110 patients (5.5%) was within the range reported by several other studies (1 – 10%) as in Indian one conducted in 2017 with a report of conversion rate of 10% [16], in USA in 2010 were the rate was 9% [17], and a local study in Iraq in 2007 [18] where the rate was 5%. In this study, GB wall thickness significantly determines the difficulty during surgery. We found that increase GB wall thickness (> 3 mm) on preoperative ultrasound which encountered in 39 patients (35.5%) in comparison to those with thin GB wall thickness (≤ 3 mm) 71 patients (64.5%) was associated with increase operative difficulty and this result was in consistent with a result found by Adwan MK et al study in 2015 [3] and with a study conducted by Sharma N et al in 2015 [19] when reported that gall bladder wall thickening can predict difficulty during cholecystectomy, we found that thickened gall bladder wall are the most accurate predictors of potential operative difficulty. GB wall thickening was a sensitive indicator of technical difficulties and the risk of conversion to open cholecystectomy. GB wall thickness is related to the inflammation and fibrosis that follow previous attach of cholecystitis and thus may reflect difficulty in delineation of the anatomy during surgery [20]. In conclusion, LC can be accomplished successfully with low morbidity in most patients with cholecystitis, those patients with increased GB wall thickness on preoperative ultrasonography are at high risk for conversion to open surgery. An accurate preoperative diagnostic tool is mandatory for planned laparoscopic GB surgery to provide information for the selection of the most appropriate approach and to avoid intraoperative difficulties and surprises.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

References

1. Litwin DE, Cahan MA. Laparoscopic cholecystectomy. Surgical Clinics of North America. 2008;88(6):1295-313.
2. Sengul S, Cetinkunar S, Ciftci E. 2. Evaluation of Potential Intraoperative Technical Difficulties with Ultrasonography before Laparoscopic

Cholecystectomy. Eur J Surg Sci. 2012;3(1):15-21.

3. Adwan MK, Alamiri MH. Relation between Gallbladder Wall Thickness, Assessed by Sonography, and Difficulties in Laparoscopic Cholecystectomy. Journal of the Faculty of Medicine. 2015;57(2):114-8.
4. Keus F, de Jong J, Gooszen H, Laarhoven CJ. Laparoscopic versus open cholecystectomy for patients with symptomatic cholecystolithiasis. Cochrane database of systematic reviews. 2006(4).
5. Lipman JM, Claridge JA, Haridas M, Martin MD, Yao DC, Grimes KL, et al. Preoperative findings predict conversion from laparoscopic to open cholecystectomy. Surgery. 2007;142(4):556-65.
6. Kania D. Ultrasound measurement of the gallbladder wall thickness in the assessment of the risk of conversion from elective laparoscopic cholecystectomy to open surgery—Olkusz county experience. Polish Journal of Surgery. 2016;88(6):334-45.
7. Qureshi TJ, Khan AU, Ashfaq A, Abid KJ. To Determine the Diagnostic Accuracy of Gallbladder Wall Thickness and Presence of Pericholecystic Fluid in Predicting the need for Conversion of Laparoscopic Cholecystectomy to Open Cholecystectomy in patients with Cholelithiasis. PAKISTAN JOURNAL OF MEDICAL & HEALTH SCIENCES. 2016;10(3):1031-4.
8. Yang T, Guo L, Wang Q. Evaluation of Preoperative Risk Factor for Converting Laparoscopic to Open Cholecystectomy: A Meta-Analysis. 2014.
9. Stanisic V, Milicevic M, Kocev N, Stojanovic M, Vlaovic D, Babic I, et al. Prediction of difficulties in laparoscopic cholecystectomy on the base of routinely available parameters in a smaller regional hospital. Eur Rev Med Pharmacol Sci. 2014;18(8):1204-11.
10. Gabriel R, Kumar S, Shrestha A. Evaluation of predictive factors for conversion of laparoscopic cholecystectomy. Kathmandu university medical journal. 2009;7(1):26-30.
11. Research NIoHOoMAo. NIH Consensus Statement: National Institutes of Health, Office of Medical Applications of Research; 1992.
12. Gul R, Dar RA, Sheikh RA, Salroo NA, Matoo AR, Wani SH. Comparison of early and delayed laparoscopic cholecystectomy for acute cholecystitis: experience from a single center. North American journal of medical sciences. 2013;5(7):414.
13. Draghi F, Ferrozzi G, Calliada F, Solcia M, Madonia L, Campani R. Power Doppler ultrasound of gallbladder wall vascularization in inflammation: clinical implications. European radiology. 2000;10(10):1587-90.
14. Jones MW, Ferguson T. Gallbladder Imaging. StatPearls [Internet]: StatPearls Publishing; 2019.
15. Pinto A, Reginelli A, Cagini L, Coppolino F, Ianora AAS, Bracale R, et al. Accuracy of ultrasonography in the diagnosis of acute calculous cholecystitis: review of the literature. Critical ultrasound journal. 2013;5(S1):S11.
16. Thyagarajan M, Singh B, Thangasamy A, Rajasekar S. Risk factors influencing conversion of laparoscopic cholecystectomy to open cholecystectomy. International Surgery Journal. 2017;4(10):3354-7.
17. Kaafarani HM, Smith TS, Neumayer L, Berger DH, DePalma RG, Itani KM. Trends, outcomes, and predictors of open and conversion to open cholecystectomy in Veterans Health Administration hospitals. The American Journal of Surgery. 2010;200(1):32-40.
18. Abdulwahab AY, Taha SA, Mutlak ST. 4-Causes and incidence of laparoscopic cholecystectomy conversion. Basrah Journal of Surgery. 2009;15(1):20-4.
19. Sharma N, Sharma D, Sukhadia M, Barolia DK. The impact of the gall bladder wall thickness on surgical management in patients undergoing cholecystectomy: a prospective study. Int J Current Microbiol Applied Sci. 2015;4(6):514-21.
20. Sharma S, Thapa P, Pandey A, Kayastha B, Poudyal S, Uprety K, et al. Predicting difficulties during laparoscopic cholecystectomy by preoperative ultrasound. Kathmandu University medical journal (KUMJ). 2006;5(17):8-11.

Evaluating the Effect of Addition of Titanium Dioxide Nanoparticle on Some Physical Properties of Flowable Composite Resin

Sazgar Srbast Qasim[1], Diyar Khalid Bakr[1], Dara H. Rasheed

[1]Hawler Medical University, College of Dentistry, conservative dentistry department/Iraq

Abstract

Objective: The flowable dental composite resins were introduced to the dental specialty because of the advantages they possess over the conventional composite resins. The use of nanotechnology in the dentistry field is one of the growing innovations in recent years. The aim of the present study was to evaluate certain physical properties of flowable dental composite after incorporation of titanium dioxide nanoparticles (TiO2NPs).

Materials and Method: In the present study, TiO2NPs at 1.25 % and 2.5% concentrations were added to flowable composite, while the unmodified composite was used as control. Then the physical properties of the control and modified composite resins, including flowablity, radiopacity and water sorption and solubility were tested. Data were analyzed with One way ANOVA, using SPSS 20.

Results: The results showed that there was statistically significant difference among the tested groups regarding flowability and radiopacity ($P<0.05$). In addition, there was no significant difference among control group and TiO2 modified groups regarding water sorption and solubility.

Conclusion: Based on the results of the present study, a flowable dental composite was successfully reinforced with TiO2. Incorporation of small weight percentages of this nanofiller exhibited properties similar to the control material regarding water sorption and solubility. The flowability was slightly reduced and radiopacity of the reinforced composites was increased, these changes were acceptable for clinical applications and below ISO standards limits.

Keywords: *Flowable Composites, Titanium Dioxide nanoparticles, flowability, radiopacity, water sorption and solubility.*

Introduction

Currently composite resins are materials used in restorative dentistry, since there are many revolution and improvements such as use of different novel particles, flowable composite resin because of their advantages over the conventional composite resins were introduced to the dental specialty. These advantages includes, simple application technique, easy handling properties, increased flowability, better adaptation to the internal cavity wall and higher elasticity, in addition, the use of flowable composites resins reduces the amount of the cavity preparation and is recommended for minimal invasive dentistry [(1)].

One of the most important innovations in the dentistry field in recent years is the use of nanotechnology. The mechanical, physical and optical properties of conventional composite resins have been improved by addition of inorganic nanoparticles such as silver, zinc, titanium dioxide and silica [(2)].

Flowability is used to describe how quickly materials flow in a certain period of time, whereas viscosity is the material's resistance to flow. Dental composites are viscoelastic materials. They share criteria of both viscous materials (e.g., oils) and elastic materials (e.g., metals).

Corresponding author:
Sazgar Srbast Qasim
Sazgar.sarbast@hmu.edu.krd

Radiopacity of dental restorative materials is an important indicator for accurate diagnosis and treatment planning. It is essential to use materials with adequate radiopacity in order to distinguish them from the natural tooth structures. Moreover, dental restorations should be radiopaque enough to detect overhanging margins, recurrent caries, restoration contour, proximal contacts [3].

The sorption and solubility properties are critical properties regarding biocompatibility concerns of releasing monomer and in relation to the stability of the composites due to degradation from the uptake of solvents and the wash-out of ingredients of materials [4]

Most of the studies involving addition of nanoparticles to dental composites resins have mainly focused on their anti-bacterial effects and the information regarding their physico-mechanical properties are limited. TiO2 are fine, non-toxic, chemically stable, and exhibits a high photocatalytic effect in addition to their high biocompatibility and pleasant color. TiO2NPs have large surface area that facilitates load transfer from resin matrix to nanoparticles thereby resulting in better mechanical properties of the reinforced composites [5].

Mohammed et al (2019) studied the effect of TiO2NPs on physico-mechanical properties of flowable dental composite resins by adding TiO2NPs at 1%, 2% and 3% to Tetric N Flow composite, while the unmodified composite was used as control. The developed composite was studies for functional and structural properties using FTIR, which indicated no change in the functional and structural characteristics [6].

The aim of the present study was to evaluate the effect of TiO2NPs, on some physical properties of flowable composite resins.

Method

In this study, the flowability, radiopacity and water sorption and solubility of a conventional flowable composite resin and composite resins reinforced with TiO2NPs were evaluated. For each test a total of 18 samples were evaluated. Commercially available flowable microhybrid composite resin (as control group) was used shade (A2). This material is based on dimethacrylate paste (Bis-GMA and Triethylene glycoldimethacrylate TEGDMA), without inorganic fillers. mixed with TiO2NPs 98% Purity and particle size of 50 nm at concentrations of 1.25% and 2.5 %.

Mixing was done by using a dental Micro motor, with a lentulo spiral-paste carrier #4 attached. The lentulo spiral was immersed in composite resin material – TiO2NPs, poured into a 2 ml pre-darkened syringe tube.

Such mixing was performed directly before preparation of samples for each test. Electronic balance was used to weight the percentage of nanoparticles, after one hour mixing; the mixture was injected into metal molds Curing was done by exposure to LED at 1,650 mW/cm2 for 40 seconds with a light guide held perpendicularly and within 2 mm of the material surface. Control samples were prepared and compared with two sets of samples with TiO2NPs at 1.25% and 2.5%.

The flowability testing method used in this study was according to ADA guidelines for evaluation of endodontic sealing materials [7]. To evaluate the flowability of the reinforced composites, a simple test using the Gillmore needle apparatus was used. The quantity of 0.1 mL of control and each reinforced composite was dispensed between two thin glass coverslips 50 x 50 mm and 1mm thick. Flowability was evaluated by comparing the composite disc diameters after they have been sandwiched between two glass coverslips, subjected to constant weight (454 g) for 30 s and light cured for 40 s.

For radiopacity test 6 discs 15±1mm in diameter and 1± 0.1mm thick were prepared for each group. The specimens and the aluminum stepwedge were placed in the center of the film and irradiated with X-rays at (65±5) kV at a target film distance of 400 mm for 0.4 seconds at 10 mA. After developing and fixing the film, the density of the image of the specimen was compared with that of the aluminum standard using the densitometer. For Water sorption and solubility test 6 discs 15±1mm in diameter and 0.5± 0.1mm thick samples were prepared for each group using a metal molds and tested according to ADA specification [8]

Statistical Analysis

The statistical analysis was performed on SPSS program version 20.0, the descriptive statistic was done for tested groups, for verification the difference among groups analysis of variance (One-way ANOVA) with Tukey post-test.

Results

Flowability test

Table 1 present the means and standard deviations of flowability values in conventional composite resin and composite resins reinforced with TiO2NPs at different percentages.

Based on table 1, the Flowability of control group and 1.25% and 2.5% groups were 2.6933 cm, 2.6083 cm and 2.5067 cm respectively, The Results of Table 1 revealed significant differences among the tested groups. But the difference is acceptable within the ISO standard.

Table 1: Mean values (mm), standard deviations, standard errors and 95% confidence intervals for Flowability data.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
(C)	6	2.6933a	.00816	.00333	2.6848	2.7019	2.68	2.70
1.25%	6	2.6083b	.00983	.00401	2.5980	2.6187	2.60	2.62
2.5%	6	2.5067c	.01211	.00494	2.4940	2.5194	2.50	2.53
Total	18	2.6028	.07910	.01864	2.5634	2.6421	2.50	2.70

Note: Means with different letter indicates statistically significant difference (P<0.05).

Radiopacity test

Radiopacity mean values of the tested materials are presented in Table (2). As recommended by ISO [9]. 2.5% group showed the highest radiopacity values 2.4760 E.q. Al thickness/mm mean value followed by 1.25% group of 2.3900 E.q. Al thickness/mm mean value of radiopacity, and 2.2833 E.q. Al thickness/mm mean value for Control group with statistical significant difference among the tested groups.

Table 2: Mean values (mm), standard deviations, standard errors and 95% confidence intervals for Radiopacity.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
control	6	2.2833a	.00816	.00333	2.2748	2.2919	2.27	2.29
1.25%	6	2.3900b	.00894	.00365	2.3806	2.3994	2.38	2.40
2.5%	6	2.4760c	.01131	.00462	2.4641	2.4879	2.46	2.49
Total	18	2.3831	.08159	.01923	2.3425	2.4237	2.27	2.49

Note: Means with different letter indicates statistically significant difference (P<0.05).

Water Sorption test

Water sorption mean values are presented in Table (3) revealed no significant differences among the tested groups. Control group showed the highest Water sorption values 1.456283 µg/mm³ mean value, followed by 1.25% group with -1.029533 µg/mm³ mean value Water sorption, and .802833 µg/mm³ mean value of 2.5% group but the difference was not significant statistically.

Table 3: Mean values (mm), standard deviations, standard errors and 95% confidence intervals for Water sorption data test.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
control	6	-1.456283a	1.2018703	.4906615	-2.717569	-.194998	-2.8037	.8131
1.25%	6	-1.029533a	1.3579421	.5543775	-2.454606	.395539	-1.8691	1.6260
2.5%	6	-.802833a	1.6504075	.6737760	-2.534830	.929163	-1.6260	2.5000
Total	18	-1.096217	1.3586916	.3202467	-1.771878	-.420555	-2.8037	2.5000

Note: Means with same letter indicates statistically no significant difference.

Water Solubility

Solubility mean values are presented in Table (4). Test results revealed no significant differences among the tested groups. Control group showed the highest solubility values 2.516583 µg/mm³ mean value, followed by 1.25% group with 2.048467µg/mm³ mean value solubility, and 1.386167µg/mm³ mean value of 2.5% group with no statistical significant difference among the tested groups.

Table 4: Mean values (mm), standard deviations, standard errors and 95% confidence intervals for Water solubility data test.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
control	6	2.516583a	.9013909	.3679913	1.570632	3.462535	.9345	3.2601
1.25%	6	2.048467a	1.1576720	.4726176	.833564	3.263369	.8130	3.8530
2.5%	6	1.386167a	1.1753861	.4798494	.152675	2.619659	.0000	3.4390
Total	18	1.983739	1.1257010	.2653303	1.423941	2.543537	.0000	3.8530

Note: Means with same letter indicates statistically no significant difference.

Discussion

The flowability of floweble composite resin decreased slightly with addition of TiO2NPs at 1.25% and 2.5% and the results are within the ISO standards, these results are in agreement with previous study reported that it is possible to increase the nanofiller content due to their small particle sizes. This will result in minimum reduction in flowability as well as improvement in the physical properties of the nano-composites [10].

In the present study the most commonly used monomer) BisGMA and TEGDMA were used, The advantages of using BisGMA over other monomers are less shrinkage, higher modulus and reduce toxicity due to its lower volatility and diffusivity into tissue. Although, BisGMA possesses high strength and hardness, the drawback of this monomer is its high viscosity (low flow), because of hydrogen bonding interaction that occur between hydroxyl groups, which limits the incorporation of inorganic fillers and hence a low degree of conversion,Thus BisGMA diluted with other low-viscosity (high flow) monomer such as trimethylene-glycol-dimethacrylate (TEGDMA) [11] to enhance flowability of the used composite that allow incorporation of TiO2NPs that resulted in a little reduction of flowability of the tested flowable composite resin and the changes are within accepted range of ISO standards

The radiopacity of a restorative material is an important parameter for accurate diagnosis and treatment planning. Success or failure of the restorative material is highly dependent on radiographs. Radiopacity of composite restorations has an important role in detecting recurrent caries and distinguishing the restorations from the tooth structures [12].

The 2.5% composite showed a higher value of radiopacity. Followed by 1.25% group Al, and control group with statistical significant difference among the tested groups and met the ISO standard for dental materials radiopacity. All of them had radiopacity greater than enamel (1.77 – 2 mm Al). These results agree with previous studies that recommended that the composite radiopacity should be equal to or greater than that of the enamel [13].

The atomic number of the elements is the most important factor affecting the radiopacity of dental materials [14]. Radiopacity of dental composites can be increased by incorporating a higher percentage of fillers with high atomic numbers [15], TiO2NPs atomic number is 22 and density of 4.506 g/^{cm3} which is considered as a high atomic number and this explains the results of this study in which radiopacity of tested material increased with increasing percentage of TiO2NPs from 0% control group to 1.25% and 2.5 %..

The sorption and solubility properties are important regarding biocompatibility concerns of releasing monomer and in relation to the stability of the composites by avoiding degradation from the uptake of solvents and the wash-out of ingredients of materials [16]. According to ISO standard [17], the maximum acceptable values of sorption and solubility for polymer-based restorative materials are 40 μg/mm3 and 7.5 μg/mm3 respectively. Sorption and solubility values for all samples were below the ISO standards limits so all investigated materials met the requirements of the ISO standard. The decreasing sorption and solubility with TiO2 content was not statistically significant. These results are in agreement with Robert et al [18] study who studied the effect of nanofillers on water sorption and solubility and concluded that the addition of nanofillers at low concentrations not change water sorption and solubility significantly since at lower concentrations there is no agglomeration of 50 nm TiO2NPs.

The improvement of both water sorption and solubility after addition of TiO2NPs might be attributed to numerous explanations such as nanofillers are insoluble in water so that the addition of TiO2NPs to the microhybrid flowable composite resin declines the solubility of composite resin [19].

Furthermore, titanium coupling agent incorporated in salinized TiO2NPs expands the adhesion between both resin matrix and filler particles which enhances composite resin properties and declines its water sorption and solubility [20].

Moreover, the reaction between resin (polar nature) and nanofillers certainly induce replacing the hydrophilic resin and minimizing the water uptake by decreasing this polarity through utilizing most active sites in the molecules of momomers, so the diffusivity of water particles through this material is greatly declined [21].

Conclusion

Based on the results, it appears incorporation of low concentration of nanofillers into conventional composite resins did not result in any changes in their water sorption

and solubility; however, flowability and radiopacity of flowable were changed but the results are acceptable withen ISO standards .Therefore, it is suggested to add small amount of nano-fillers to composite resins to prevent problems such as discoloration of composite resin or other possible changes in other physical and chemical properties.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

References

1. J. Payne, The marginal seal of Class II restorations: Flowable composite resin compared to injectable glass ionomer. Journal of Clinical Pediatric Dentistry 23,123 (1999).
2. S. E. Elsaka, I. M. Hamouda, and M. V. Swain, Titanium dioxide addition to a conventional glass-ionomer restorative:Influence on physical and antibacterial properties. Journal of Dentistry 39, 589 (2011).
3. Tveit AB, Espelid I. Radiographic diagnosis of caries and marginal defects in connection with radiopaque composite fillings. Dental Materials. 1986;2(4):159-62.
4. Ferracane, J.L. Resin-based composite performance: Are there some things we can't predict? Dent. Mater.Off. Publ. Acad. Dent. Mater. 2013, 29, 51–58. [CrossRef] [PubMed
5. B. Wetzel, P. Rosso, F. Haupert, and K. Friedrich, Epoxy nanocomposites–fracture and toughening mechanisms. Engineering Fracture Mechanics 73, 2375 (2006).
6. Mohammed Al Jafary, Mohamed Ibrahim Hashem, Majdah A. Al Khadhari, Sara Abdullah Alshammmery, and Mansour K. Assery, Effect of Nanoparticles on Physico-Mechanical Properties of Flowable Dental Composite Resins; Science of Advanced Materials. 2019;Vol. 11, pp. 1–8.
7. ADA Professional Product Review. Laboratory testing methods: Endodontic filling and sealing materials. 2008; 3(4).
8. American National Standards/American Dental Association specification no. 27 for resin-based filling materials. 1993; Chicago: American Dental Association,Council on Scientific Affairs.
9. International Standardization Organization, ISO 4049–2000. Dentistry-polymer based filling restorative and luting materials; 7.10 depth of cure, Class 2 materials. 3rd ed. Geneva: ISO; 2000.
10. Bayne SC, Thompson JY, Swift EJ,Jr, Stamatiades P, Wilkerson M. A characterization of first-generation flowable composites. J Am Dent Assoc. 1998;129(5):567-77.]
11. Manal Dafar. "Reinforcement of Flowable Dental Composites with Titanium Dioxide Nanotubes" Master thesis, 2014.
14. Chen M-H. Update of Dental Nanocomposites. Journal of Dental Research. 2010;89(9):549-560.
12. Oztas B, Kursun S, Dinc G, Kamburoglu K. Radiopacity evaluation of composite restorative resins and bonding agents using digital and film x-ray systems. Eur J Dent. 2012; 6(2):115-22.].
13. Bouschlicher MR, Cobb DS, Boyer DB. Radiopacity of compomers, flowable and conventional resin composites for posterior restorations. Oper Dent. 1999;24(1):20-25.
14. Watts DC, McCabe JF. Aluminium radiopacity standards for dentistry: An international survey. J Dent. 1999; 27(1):73-78
15. Sabbagh J, Vreven J, Leloup G. Radiopacity of resin-based materials measured in film radiographs and storage phosphor plate (digora). Oper Dent. 2004; 29(6):677-84.
16. Ferracane, J.L. Resin-based composite performance: Are there some things we can't predict? Dent. Mater. Off. Publ. Acad. Dent. Mater. 2013; 29, 51–58. [CrossRef] [PubMed]
17. (International Standards Organazation) 4049:2009 (E) Dentistry- polymer-based restorative materials. Geneve, Switzerland
18. Robert Stencel , Jacek Kasperski , Wojciech Pakieła , Anna Mertas , Elzbieta Bobela, Izabela Barszczewska-Rybarek and Grzegorz Chladek . Properties of Experimental Dental Composites Containing Antibacterial Silver-Releasing Filler, Materials 2018, 11, 1031; doi:10.3390/ma11061031
19. Noor AS. Evaluation of AL2O3 on thermal conductivity of acrylic resin denture base and some other properties. College of dentistry. J Univ Baghdad 2010; 23:12–19.

20. Elshereksi NW, Ghazali MJ, Muchtar A, Azhari CH. Perspectives for titanium-derived fillers usage on denture base composite construction: a review article. Adv Mat Sci Eng 2014; 1:1–14.

21. Jadhav R, Bhide SV, Prabhudesai PS. Assessment of the impact strength of the denture base resin polymerized by various processing techniques. Indian J Dent Res 2013; 24:19–25.

Antimicrobial Activities of Green Biosynthesized Iron Oxide Nanoparticles Using *F. Carica* Fruit Extract

Nada Khudair Abbas[1], Azhar A. F. Al- Attraqchi[2], tutor Jenan Hussien Taha[3]

[1]Department of Physics, College of Science, University of Baghdad, Baghdad, Iraq, [2]Department of Microbiology, College of Medicine, University of Al- Nahrain, Baghdad, Iraq, [3]Physiology and Medical Physics Department, College of Medicine, University of Al- Nahrain, Baghdad, Iraq

Abstract

In the current study, iron oxide nanoparticles (IO NPs) were synthesized via modified green synthesis technique using *F. carica* fig extract as reducing agent. Furthermore, the microstructural properties of the synthesized IO NPs have been thoroughly elucidated. In details, the acquired NPs diameter was found to be in the range of 11-29 nm and of root mean square (RMS) of 0.64 nm using TEM and AFM techniques, respectively. Consequently, the antifungal and antibacterial activities of the synthesized IO NPs were screened against *Candida* and *Aspergillus* species as well as Gram-positive *Staphylococcus aureus* and Gram-negative *Acinetobacter* species, respectively. The presented IO NPs play an active role in the antimicrobial activities evidencing the well-organized materials system for biomedical applications.

Keywords: *Iron oxide NPs, antifungal, antibacterial, F. carica extract*

Introduction

Metal oxide NPs are of great importance due to a number of unique properties such as high surface-to-volume ratio, easy separation features, and low toxicity [(1)]. IO NPs have attracted a great significance in the field of nanoscience and nanotechnology [(2, 3)]. In conjunction with IO NPs, several applications have been proposed in the area of optoelectronics, catalysis, self-powered smart window, lithium ion batteries and diagnostic biological probes [(4-8)]. IO NPs are extensively applied in the biomedicine field due to their low toxicity and biocompatibility. Due to the electrostatic feature of the IO NPs, they are easily interact with fungal, and bacterial living-cell membranes [(9)]. This property allows IO NPs to harms the cell membranes of fungi or bacteria as well as inducing the toxic oxidative stress via free radical formation [(10)]. Antimicrobial activities of IO NPs are, therefore, dependent on three essential features namely concentration of the culture media and most importantly stability [(9, 10)].

Corresponding author:
Jenan Hussien Taha
asjenan@gmail.com

In this attempt, variety of approaches have been anticipated for the synthesis of IO NPs. Among these are the well-known physical and chemical methods in which preferred NPs properties can be acquired. Particularly, these methods are electrodeposition [(11)], conventional heating [(12)], hydrothermal [(13, 14)], wet oxidation [(15)], laser ablation [(16)], co-precipitation [(17, 18)], and anodization [(19)]. However, these techniques are presented with several draw-backs such as the use of non-biodegradable stabilizing agents and toxic chemicals, and hypothetically harmful to the well-being organisms and the surrounding environment. The green synthesis technique, which is utilized in the current study, has revealed considerable advances such as environmentally friendly, unpresented toxic chemicals, and low energy and temperature conditions [(20)]. Green synthesis technique evolves the use of naturally existing resources such as plants extracts as reducing fuel [(21)]. Therefore, this manuscript reports a modified green synthesis technique of IO NPs in which *F. carica* fig extract is used as a reducing agent. Additionally, the antifungal activity against *Candida* and *Aspergillus* species and antibacterial activity against Gram-positive *S. aureus* and Gram-negative *Acinetobacter* species were thoroughly investigated.

Materials and Method

Plant collection and extraction

Dried *F. carica* (common fig) was purchased from local market in Baghdad, Iraq. Consequently, the collected fruit was washed thoroughly; afterward, 10 gm of the washed fruit was blended alongside 150 mL of deionized-distilled water (DDW). The resultant mixture was subjected to heating process at 100 °C in a ventilation oven and then cooled down to room temperature. Finally, the solution mixture was filtered using chromatography paper (Whatman No.1) and incubated at room temperature for further use.

Synthesis of IO NPs

In a typical laboratory route, specific amount (0.1, 0.14 and 0.18 molar) of iron^{III} chloride hexahydrate ($F_2Cl_3.6H_2O$) was liquefied in 100 mL of DDW under stirring rate of 800 r.p.m. for 15 minutes at RT. The consequential mixture was then mixed with fig extract (1:1) under stirring rate of 600 r.p.m. for 2 h at 50 °C [(1)]. The first indication of the NPs occurring was observed upon color changing of the solution, whereby a light brown color was noticed, as presented in figure (1). The acquired solution was then washed and centrifuged at 4000 r.p.m. for 20 minutes. Hereinafter, the attained residual was dried for 6 h at 60 °C and later grinded using mortar and pestle to obtain a fine NPs powder.

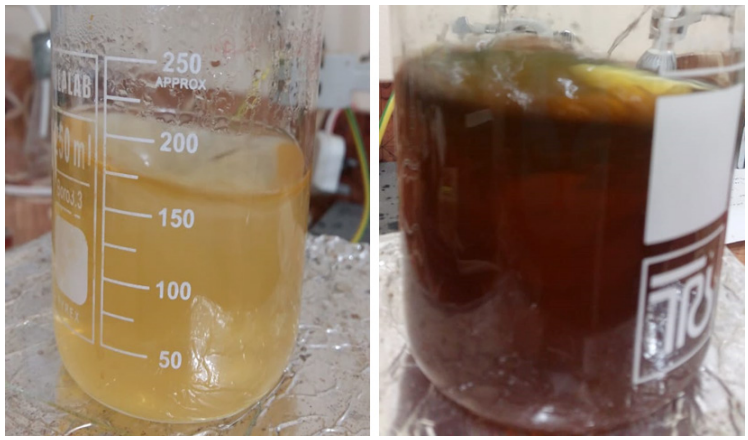


Figure (1): Color changing process of IO NPs.

Characterization

The microstructural properties of the prepared NPs were examined using Shimadzu X-ray Diffractometer (XRD-6000) with wavelength of 1.541 A° and Cu-Kα radiation. In the meanwhile, compact char surface was investigated using Fourier Transform Infrared Spectroscopy (FT-IR, Thermo Nicolet Nexus) ranging from 400 to 4000 cm^{-1}. Furthermore, the optical properties of the prepared NPs were recorded on Shimadzu UV-1800 UV-Vis spectrophotometer. Atomic Force Microscopy (SPM AA3000-AFM) and TECNAI F-30 TEM were engaged for the morphological and nanoparticle size investigations.

Evaluation of antifungal and antibacterial activities

The antifungal activity of the synthesized NPs was monitored using agar well diffusion procedure against two fungal species which are *Candida* and *Aspergillus* species [(22)]. Agar petri dish, which was used in this study as a culture media holder, was systematically swabbed with sterile cotton swab in which a 30 ml of 24 h Sabouraud′s dextrose was used for each fungal species. Continuously, wells were made in the pre-solidified agar plates with the help of 5 mm cork bor-er. Variety of the synthesized NPs concentrations (0.75, 1.5, 3, 6, 12 and 24 mg/ml) were sonicated with DDW and then used to evaluate the antifungal activity. Concurrently, Negative and positive control against the fungal pathogens was exhibited using DDW and antibiotics. Hereinafter, the cultured agar plates were incubated at 35 °C for 48 h, while the inhibition zones were measured in millimeter.

As for the IO NPs antibacterial activity, similar route to the aforementioned antifungal was repeated. However, the antibacterial activity was screened against Gram-positive *S. aureus* and Gram-negative *Acinetobacter* species using 30 ml of 24 h Blood agar culture media. In

this experiment, the positive and negative controls used were antibiotics and DDW and the antibacterial activity was later proceeded to an incubation procedure for 24 h at 35℃. It is worth mentioning that the concentrations of IO NPs utilized for the antibacterial activity are 0.5, 1, 2, 4, and 8 mg/ml.

Results and Discussion

The XRD patterns of the synthesized NPs are presented in figure (2, a). Generally, the intensity of the diffraction peaks augmented with increasing IO NPs concentrations which in turn indicates higher crystallinity at high concentrations. As depicted in the figure, eight different pronounced peaks were acquired corresponding to (110), (120), (211), (10-1), (202), (211), (312), and (310) planes and diffraction angle of 2θ = 21.9°, 26.6°, 33.2°, 35.6°, 49.5°, 50.4°, 54°, and 62.4°, respectively (Card No. 96-901-1413) [1]. The (110), (120), and (211) planes mainly belong to FeO_2 phase, while other planes, (211), (10-1), (202), (312), and (310), are corresponded to Fe_2O_3 phase (Card No. 96-900-9783) [9].

Figure (2, b) illustrates the FT-IR curve of the synthesized NPs with concentration of 0.14 M. It is clear to be noticed that characteristic peaks at 3848, 3737, and 3416 cm^{-1} are mainly attributed to the O-H stretching bonds. In the meanwhile, peak at around 2925, cm^{-1} is corresponded to C-H stretching characteristic. Two pronounced peaks at 2381, and 2310 cm^{-1} are designated to O=C=O stretching. Furthermore, additional peaks at 1737, and 1638 cm^{-1} were observed which are due to C=O stretching, while peak at 1542 cm-1 is found to be in accordance with C=C stretching [1, 23]. Finally, Fe-O vibrations namely 1098, 795, 506 and 463 cm^{-1} are assigned to the IO NPs [23].

The UV-vis spectra of the synthesized IO NPs are demonstrated in figure (2, c), which exhibited broad bands and cut-off phenomenon at 360 nm. Furthermore, as illustrated in the figure, there is a decrease in the mentioned phenomenon cut-off towards higher wavelength as the concentration enlarged; this could be attributed to the lattice defects in the prepared NPs matrix [9].

Figure (2, d) demonstrates two dimensions and three dimensions AFM images of the synthesized NPs with a concentration of 0.14 M and scanning area of 2 µm. In general, the formed NPs exhibited a vertically aligned NPs with regular spherical shape and homogenous distribution. The average diameter and RMS were found to be 84.4 and 0.64 nm, respectively. In the meanwhile, the average surface roughness was found to be 0.54 nm; this indicates a pronounced rough surface which in turn reveals high electrochemical performance [24].

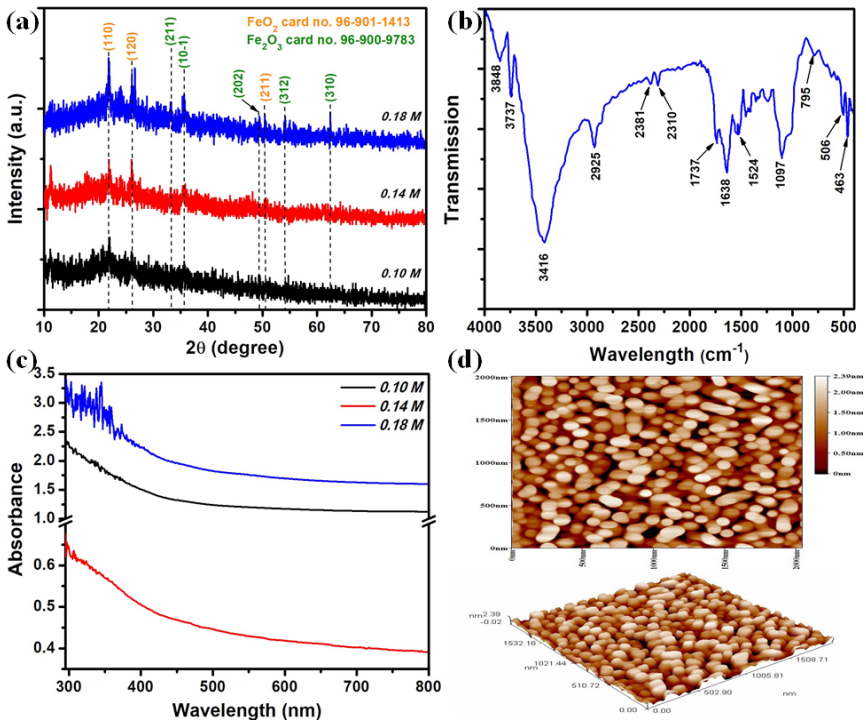


Figure (2): (a) XRD patterns, (b) FT-IR spectra, (c) UV-vis spectra, and (d) AFM of 2D and 3D images of the prepared NPs.

The TEM image of IO NPs is presented in figure (3, a and b). Generally, the synthesized NPs revealed almost a spherical structure with average diameter of 19 nm. From the figure, it also can be observed that a uniform IO NPs distribution which found to be in good agreement with the AFM findings. Furthermore, figure (3, b) shows the NPs diameter distribution which was found to be in the range of 11-29 nm.

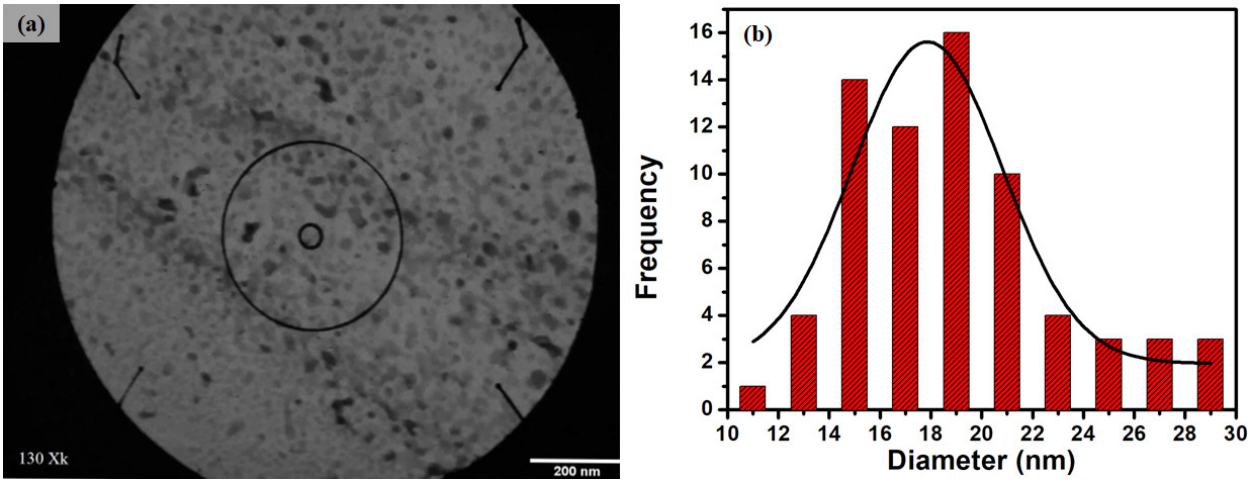


Figure (3): IO NPs with 0.14 M concentration (a) TEM image (b) diameter range distribution.

The antifungal activity of the green synthesized NPs against *Candida* and *Aspergillus* species are shown in figure (4, a). As presented in the figure, an observable inhibition zone increment in both kind of fungi species can be clearly seen as the concentration of the IO NPs increased, this indicates the active role of the synthesized NPs as an antifungal. This can be explained by the superior features of IO NPs such as large surface area, and small particle size as compared to their bulk nature [25]. Although, the inhibition zones in the case of *Candida* demonstrated larger inhibition diameter, lower concentrations of the used NPs were found to be active in the case of *Aspergillus*. The maximum inhibition zone diameters were found by the highest IO NPs concentration 24 (mg/ml) against *Candida* (35 mm) followed by *Aspergillus* (33 mm). In contrast, inhibition zone diameters of 10 and 15 mm against *Candida* and *Aspergillus* with IO NPs concentration of 3 mg/ml, respectively. Similarly, at concentration of 1.5 mg/ml, the antifungal activity was only exhibited against *Aspergillus*. This may be due the differences in the *Candida* species' cell wall structure which consists of high chitin as a yeast as compared to the moldy in *Aspergillus* species [26], this in turn leads to different sub-capability of each towards the tested nanoparticles.

Figure (4, b) presents the antibacterial activity of IO NPs against Gram-positive *S. aureus* and Gram-negative *Acinetobacter* species. Generally, increasing the concentration of IO NPs exhibited higher antibacterial activity. This could be attributed to the metallic NPs accumulation in the living cell-membranes which in turn releases cellular compounds as previously reported by other researchers [27]. It is a necessity to be stated that concentration as low as 0.5 mg/ml exhibited null activity against both species. However, higher concentrations of IO NPs displayed advanced inhibition zones in both species cases. Continuously, the acquired inhibition zones against Gram-negative *Acinetobacter* bring about more diameter range as compared to Gram-positive *S. aureus* at all concentrations. Inhibition zones of 25 and 19 mm in diameter against Gram-negative *Acinetobacter* and Gram-positive *S. aureus* were screened with IO NPs concentration of 8 mg/ml, respectively. This may be due to the difference in susceptibilities property of each bacterial species towards IO nanoparticles comes from the differences in the structure of cell wall of each bacteria. In details, the Gram-positive *S. aureus* species has thicker peptidoglycan and thus demonstrate higher resistance in contrast to Gram-negative *Acinetobacter*. As observed in table (1 and 2), the synthesized NPs demonstrated higher antibacterial and antifungal effects than standard antibiotics

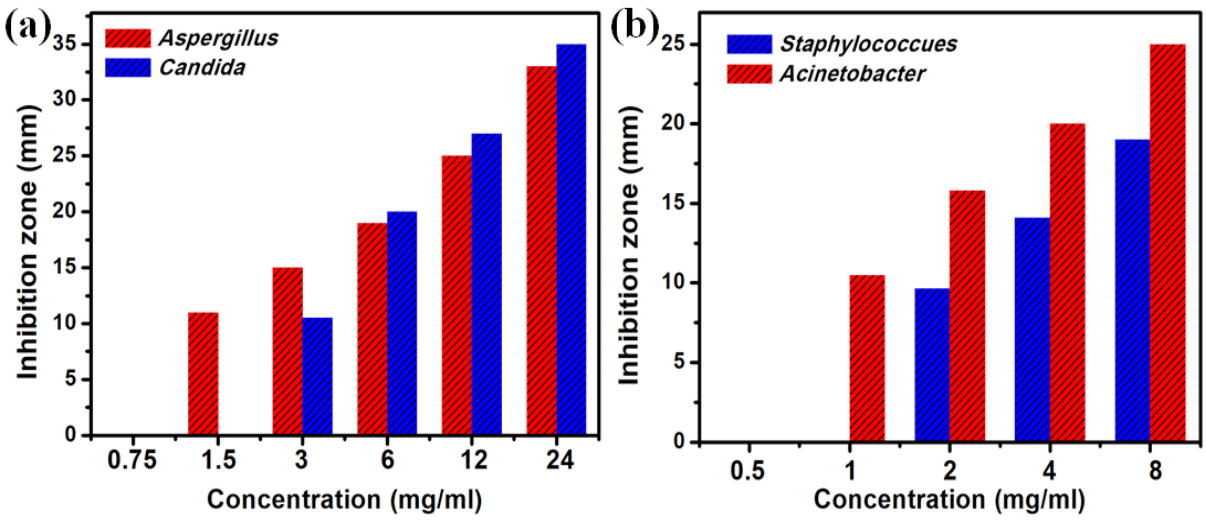


Figure (4): Inhibition zones of IO NPs; (a) fungi and (b) bacteria.

Table (1): Antibiotics zone of inhibition diameters against fungi.

fungal	inhibition zone (mm)				
	KCA (10 µg)	NY (100un)	AMB (20µg)	FCN (10µg)	DDW
candida spp.	34	14	11.4	22	0
Aspergillus spp.	21	16	12	0	0

Table (2): Antibiotics zone of inhibition against bacteria.

bacterial	inhibition zone (mm)							
	CRO (30 µg)	AK (30µg)	SAM (20µg)	TS (25µg)	CD (2µg)	CIP (5µg)	ATH (15µg)	DDW
S. aureus	26	17.5	14.5	13.5	0	0	0	0
Acinetobacter baumanii	0	0	0	0	0	0	0	0

Conclusion

IO NPs were successfully synthesized using *F. carica* fig extract. Additionally, the microstructural properties of the prepared NPs were illustrated using XRD, FT-IR, UV-vis, AFM and TEM techniques. Simultaneously, the synthesized NPs were evaluated for their antifungal and antibacterial activities against *Candida* and *Aspergillus* species as well as *S. aureus* and *Acinetobacter* species, respectively. It was found that *Aspergillus* species is more sensitive to the synthesized NPs rather than *S. aureus,* shedding the light towards this technique which may be useful for the treatment of this aggressive multidrug resistant bacteria.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

References

1. Demirezen DA, Yıldız YŞ, Yılmaz Ş, Yılmaz DD. Green synthesis and characterization of iron oxide nanoparticles using Ficus carica (common fig) dried fruit extract. Journal of bioscience and bioengineering. 2019;127(2):241-5.
2. Naseem T, Farrukh MA. Antibacterial activity of green synthesis of iron nanoparticles using Lawsonia inermis and Gardenia jasminoides leaves extract. Journal of Chemistry. 2015;2015.
3. Salih EY, Sabri MFM, Hussein MZ, Sulaiman K, Said SM, Saifullah B, et al. Structural, optical and electrical properties of ZnO/ZnAl 2 O 4 nanocomposites prepared via thermal reduction approach. Journal of Materials Science. 2018;53(1):581-90.
4. Kouhbanani MAJ, Beheshtkhoo N, Taghizadeh S, Amani AM, Alimardani V. One-step green synthesis and characterization of iron oxide nanoparticles using aqueous leaf extract of Teucrium polium and their catalytic application in dye degradation. Advances in Natural Sciences: Nanoscience and Nanotechnology. 2019;10(1):015007.
5. Aslam MK, Shah SSA, Najam T, Li S, Chen C. Decoration of cobalt/iron oxide nanoparticles on N-doped carbon nanosheets: Electrochemical performances for lithium-ion batteries. Journal of Applied Electrochemistry. 2019;49(4):433-42.
6. Swidan MM, Khowessah OM, El-Motaleb MA, El-Bary AA, El-Kolaly MT, Sakr TM. Iron oxide nanoparticulate system as a cornerstone in the effective delivery of Tc-99 m radionuclide: a potential molecular imaging probe for tumor diagnosis. DARU Journal of Pharmaceutical Sciences. 2019;27(1):49-58.
7. Salih EY, Sabri MFM, Tan ST, Sulaiman K, Hussein MZ, Said SM, et al. Preparation and characterization of ZnO/ZnAl 2 O 4-mixed metal oxides for dye-sensitized photodetector using Zn/Al-layered double hydroxide as precursor. Journal of Nanoparticle Research. 2019;21(3):55.
8. Bashir MBA, Said SM, Sabri MFM, Miyazaki Y, Shnawah DA, Shimada M, et al. In-Filled La 0.5 Co 4 Sb 12 Skutterudite System with High Thermoelectric Figure of Merit. Journal of Electronic Materials. 2018;47(4):2429-38.
9. Ismail RA, Sulaiman GM, Abdulrahman SA, Marzoog TR. Antibacterial activity of magnetic iron oxide nanoparticles synthesized by laser ablation in liquid. Materials Science and Engineering: C. 2015;53:286-97.
10. Iwamoto T, Ishigaki T, editors. Fabrication of iron oxide nanoparticles using laser ablation in liquids. Journal of Physics: Conference Series; 2013: IOP Publishing.
11. Aghazadeh M, Karimzadeh I. One-pot electro-synthesis and characterization of chitosan capped superparamagnetic Iron oxide nanoparticles (SPIONs) from ethanol media. Current Nanoscience. 2018;14(1):42-9.
12. Zhu J, Yang Z, Li X, Qi S, Jia M. Application of microwave heating with iron oxide nanoparticles in the in□situ exploitation of oil shale. Energy Science & Engineering. 2018;6(5):548-62.
13. Pandi K, Viswanathan N, Meenakshi S. Hydrothermal synthesis of magnetic iron oxide encrusted hydrocalumite-chitosan composite for defluoridation studies. International journal of biological macromolecules. 2019;132:600-5.
14. John Vagabond's Physics and Chemistry Blog. HOW DO PHOTONS INTERACT WITH MATTER? SIMPLE SCATTER, COMPTON SCATTER, PAIR PRODUCTION 2014, April 14. Available from: https://johnvagabondscience.wordpress.com/tag/pair-production/.
15. Elnabawy HM, Casanova-Chafer J, Anis B, Fedawy M, Scardamaglia M, Bittencourt C, et al. Wet chemistry route for the decoration of carbon nanotubes with iron oxide nanoparticles for gas sensing. Beilstein journal of nanotechnology. 2019;10(1):105-18.
16. Zhang D, Choi W, Oshima Y, Wiedwald U, Cho S-H, Lin H-P, et al. Magnetic Nanoblends Fe@ FeO Synthesized x Single-Crystal by Femtosecond Laser Ablation of Fe in Acetone. Laser-Based Nano Fabrication and Nano Lithography. 2018:107.
17. Salih EY, Abbas Z, Al Ali SHH, Hussein MZ. Dielectric behaviour of Zn/Al-NO3 LDHs filled with polyvinyl chloride composite at low microwave frequencies. Advances in Materials Science and Engineering. 2014;2014.
18. Salih EY, Sabri MFM, Sulaiman K, Hussein MZ, Said SM, Usop R, et al. Thermal, structural, textural

and optical properties of ZnO/ZnAl2O4 mixed metal oxide-based Zn/Al layered double hydroxide. Materials Research Express. 2018;5(11):116202.

19. Lucas-Granados B, Sánchez-Tovar R, Fernández-Domene RM, Garcia-Anton J. Influence of electrolyte temperature on the synthesis of iron oxide nanostructures by electrochemical anodization for water splitting. International Journal of Hydrogen Energy. 2018;43(16):7923-37.
20. Kavitha K, Baker S, Rakshith D, Kavitha H, Yashwantha Rao H, Harini B, et al. Plants as green source towards synthesis of nanoparticles. Int Res J Biol Sci. 2013;2(6):66-76.
21. Groiss S, Selvaraj R, Varadavenkatesan T, Vinayagam R. Structural characterization, antibacterial and catalytic effect of iron oxide nanoparticles synthesised using the leaf extract of Cynometra ramiflora. Journal of Molecular Structure. 2017;1128:572-8.
22. Pérez Rodríguez C, Paul M, Bazerque P, Perez-Eid C. An Antibiotic assay by the agar well diffusion method. 1990.
23. Namduri H, Nasrazadani S. Quantitative analysis of iron oxides using Fourier transform infrared spectrophotometry. Corrosion Science. 2008;50(9):2493-7.
24. Soomro RA, Sherazi SH, Memon N, Shah M, Kalwar N, Hallam KR, et al. Synthesis of air stable copper nanoparticles and their use in catalysis. Adv Mater Lett. 2014;5(4):191-8.
25. Khan I, Saeed K, Khan I. Nanoparticles: Properties, applications and toxicities. Arabian Journal of Chemistry. 2019;12(7):908-31.
26. dos Santos VM, Dorner JW, Carreira F. Isolation and toxigenicity of Aspergillus fumigatus from moldy silage. Mycopathologia. 2003;156(2):133-8.
27. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. Journal of colloid and interface science. 2004;275(1):177-82.

Molecular Detection of Antibiotics Resistance Genes in *Burkholderia Cepecia* Isolated From Diabetic Foot Infection

Maryam Riyadh Al-Muhanna[1], Mahdi Al-Ammar[1]

[1]University of Kufa-Faculty of Science-Department of Biology/Iraq

Summary

This study aimed to isolate and diagnose *Burkholderia cepetia* from clinical specimens of diabetic foot and study the resistance of bacteria to antimicrobial agent in Najaf governorate from Augest 2019 to November 2019, which includes 120 clinical specimens for both sexes with an age ranged between)35-70) years old. The diagnosis of *bacteria* isolates was based on microscopy, as well as the culture and biochemical characteristics as an initial diagnosis. The final diagnosis by the Vitek-2 compact system .

Burkholderia cepacia 8(6.6%). Antibiotic sensitivity test was examined by dick diffution method, *Burkholderia cepacia* isolates showed high level of resistance almost for all β-lactam antibiotic classes under study which included;, ceftriaxone, cefoxitin and Cefepime, with percentage of (100%); ticarcllin with clavulalnic acide ,piperacllin , ceftazidime, tobramycine, ciproflaxcin and levofloxacin with percentage of (87.5%); aztreonam and amikacine with percentage(62.5); meropenem , imipenem and gentiamycin with percentage of (37.5%).

At molecular study, the investigated the presence of antibiotic-resistant genes (blaImp, blaOxa, blaKpc,blaCTX-M) using PCR technique and electrophoresis systems. 6/8 *Burkholderia cepacia* isolates were with *blaOXA* and (6/8) of isolates carry *blaCTX-M* gene, all isolates of *Burkholderia cepacia* gave negative result of blaIMP and blaKPC gene. Finally PCR analysis showed that the integron gene was (3/8 %).

Keyword: *Bcc, Burkholderia cepacia* ; *Antibiotics Resistance; Dabetic*

Introduction

The *Burkholderia cepacia* complex (Bcc) organisms are opportunistic nosocomial pathogens capable of causing severe disease in immunocompromised individuals. Bacteria frequently employ disparate mechanisms that act synergistically to achieve elevated resistance (1).

However, these data may overestimate the occurrence of resistance in *Burkholderia cepetia* organisms as the study was carried out on patient isolates solicited because they were in fact multidrug resistant. Despite this caveat, resistance patterns, both intrinsic and acquired, must not be discounted in these organisms .The often high-level acquired or intrinsic resistance of non-enteric bacteria such as *P. aeruginosa* and *Burkholderia* species is in no small part attributable to synergy between reduced penetration into and efflux from the cell (2).

Diabetes mellitus (DM) is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerve and constitutes the most frequent diabetes-related cause of hospitalization (3).

Diabetic foot ulcers is one of the main causes of mortality and morbidity among people with diabetes. Its include an injury to all layers of skin, necrosis or gangrene that usually occur on the soles of the feet, as a result of peripheral neuropathy or peripheral arterial disease (PAD) in diabetes patients (4).

Materials and Method

specimens collection and bacterial identification

A total of 120 samples were collected from diabetic foot ulcer who attended different hospitals during the period from Augest 2019 to November 2019 in Al-Najaf provenance, sample collection include,collection 120 pus samples swab specimens from diabetic foot infection ulcer, The specimens were transported by sterile transport swabs to the department of bacteriology laboratory. Each specimen was inoculated using direct method of inoculation on culture of selective media namely MacConkey, Blood ,Mannitol agar then inoculated at 37°C for 18-24 hours [5].

DNA Extraction

Genomic DNA was extracted by using a commercial extraction system (Genomic DNA promega Kit).

Molecular Identification

Gel electrophoresis was used for detection of DNA by UV transilluminator . The PCR assay was performed to detect the antibiotic resistance gene for *Burkholderia cepacia* shown in table(2). This primer was designed by Alpha DNA company, Canada as in table (1) . Amplified products were confirmed using 1% agarose gel electrophoresis to estimate the PCR products size. The gel was stained with 4 µLof 10mg/mL ethidium bromide (Sigma, USA) and it run at 80v for 1.5h. A single band was observed at the desired position on ultraviolet light transillumintor (Cleaver, UK); bands were photographed using gel documentation system (Cleaver, UK). A 100bp ladder (Bioneer, Korea) was used to measure the molecular weights of amplified products [6].

Table (1): Primers used in this study

Primer Type	Primer Target	Primer sequence (5'-3')	Amplicon size (bp)	Reference
CTX-M	bla_{CTX-M}	**F**: SCS ATG TGC AGY ACC AGT AA **R**: CCG CRA TAT GRT TGG TGG TG	554	(7)
KPC	bla_{KPC}	**F**: ATG TCA CTG TAT CGC CGT CT **R**: TTT TCA GAG CCT TAC TGC CC	893	(8)
IMP	bla_{IMP}	**F:** TTGACACTCCATTTACDG **R:** GATYGAGAATTAAGCCACYCT	139	(9)
OXA	*blaOxa*	**F:** GGCACCAGATTCAACTTTCAAG **R:** GACCCCAAGTTTCCTGTAAGTG	564	(9)

Table (2): PCR program of *intI* primer that apply in the thermocycler

Gene	Initial denaturation	No. of cycles	Denaturation	Annealing	Extension	Final extension
blaCtxm	94 Cº for 4min.	35	94 Cº for 30Sec	63Cº for 1 min	72 Cº for 1min.	72Cº for 5min.
blaKpc	94 Cº for 5 min.	35	94 Cº for 1min	50Cº for 1min.	72 Cº for 1 min	72Cº for 10min.
blaImp	94 Cº 10 min	30	94 Cº 40Sec	55 Cº for 40Sec	72 Cº for 1 min	72 Cº for 10 min
blaOxa	94 Cº for 10 min	30	94 Cº for 40Sec	60 Cº for 40Sec	72 Cº for 1min	72 Cº for 5 min

Results and Discussion

This study was conducted on 120 specimens from dabetic foot suspected patients during the period from September 2019 to December 2019

4.9.1.2 Molecular identification of antimicrobial drug resistance of *Pseudomonas aerginosae and Burkholderia cepacia*

4.9.3.2.1.blaOXA

The result showed that the *blaOXA* resistance gene was detected in 6/8 *Burkholderia cepacia* as in figure (1) .

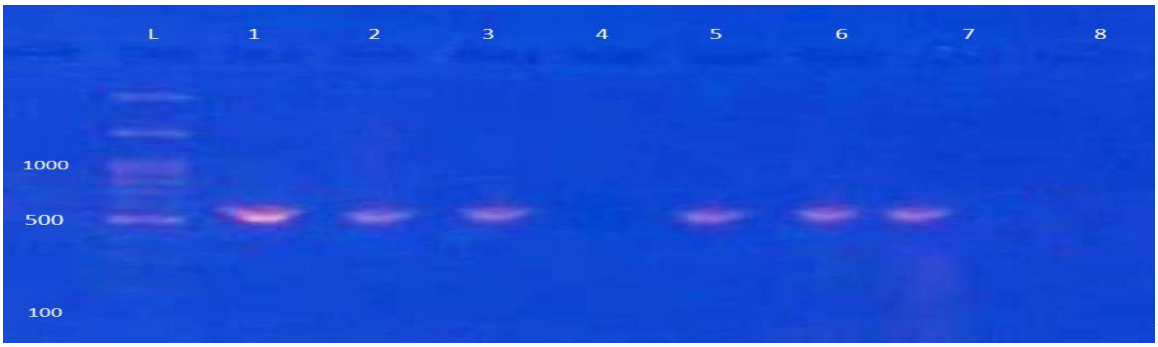


Figure (1): PCR amplification products of *Burkholderia cepacia* isolates that amplified with *blaOXA* gene primers with product 564bp.Lane (L), DNA molecular size marker (100-bp ladder), Lanes (1,2,3,5,6,7) show positive results with *blaOXA* gene.

Carbapenemases are the main mechanism by which resistance to carbapenems occurs and they belong to three of the four β-lactamase classes A, B and D . Class D carbapenemases are the OXA-β-lactamases , further subdivided into various sub-groups mainly blaOXA-23, blaOXA-24/40, blaOXA-58, blaOXA-48, blaOXA-51 and blaOXA-143 . These OXA-type β-lactamases occur widely in Acinetobacter with the most abundant being blaOXA-51, which is chromosomally encoded hence intrinsic to these species but it may confer resistance to carbapenems when its expression is up-regulated by genetic re-organization [10] Class B carbapenemases are also known as the metallo-β-lactamases (MBLs) ,they are mostly encoded by integronborne mobile gene cassettes and hence, they are transferable amongst various bacteria via horizontal gene transfer mechanisms notably conjugation . Class A carbapenemases include the Klebsiella pneumoniae carbapenemase (KPC) family that can be plasmid encoded or chromosomal [11].

4.9.3.2.2.blaIMP

The result showed negative with *blaIMP* gene of *Burkholderia cepacia* isolates . Based on recent reports, there are two major families of imported metallo--lactamases, IMP and VIM, that are carried on mobile gene cassettes inserted into integrons . Including those in this report, there are 18 variants of IMP metallo--lactamases and 11 variants of VIM metallo--lactamases [12].

The only two published reports on metallo-B-lactamases from the United States identified VIM-2 and VIM-7. Metallo-B-lactamases hydrolyze most -lactam antibiotics except aztreonam. Therefore, many pathogens that produce these enzymes at high levels are resistant to the majority of -lactam antibiotics, including the carbapenems. The first report of an imported metallo-B-lactamase described a *Pseudomonas aeruginosa* isolate obtained from a Japanese patient in 1988 . Since then, the occurrence of mobile genetic elements encoding metallo-B-lactamases has extended beyond *P. aeruginosa* to include many types of gram-negative organisms distributed throughout the world . Areas which have reported these types of isolates include several countries in Asia and Europe; the Americas, including Brazil, Canada, and the United States; and Australia [12].

4.9.3.2.3.blaKPC

All *Burkholderia cepacia* isolates give negative result with *blaKPC* gene .

The blaKPC genes that encode KPCs are present on transferable plasmids and are flanked by transposable elements, thus allowing for the gene to move from plasmid to the bacterial chromosome and back . [13]. All the carbapenem resistant isolates showed 100%

resistance to ampicillin, cotrimoxazole, all 4 generations of cephalosporins and piperacillin tazobactam. The resistance to aminoglycoside antibiotics varied from 33% for amikacin to 94% to tobramycin . In the present study, *Klebsiella* showed a 77% resistance to imipenem and 96% resistance to meropenem, while *E coli* showed 67% resistance to imipenem and 95% resistance to meropenem, blaKPC gene Detection in Clinical Isolates of Carbapenem Resistant Enterobacteriaceae were MHT negative. They may have developed a different resistant mechanism other than carbapenemase production. Resistant to both imipenem and meropenem is a strong indicator of carbapenemase production rather than resistance to either one of the carbapenems, as this may imply a different resistance mechanism [14].

4.9.3.2.4.blaCTX-M gene

The result showed that the blaCTX-M resistance gene was detected in and 6/8 *Burkholderia cepacia* as in figure,(2) .

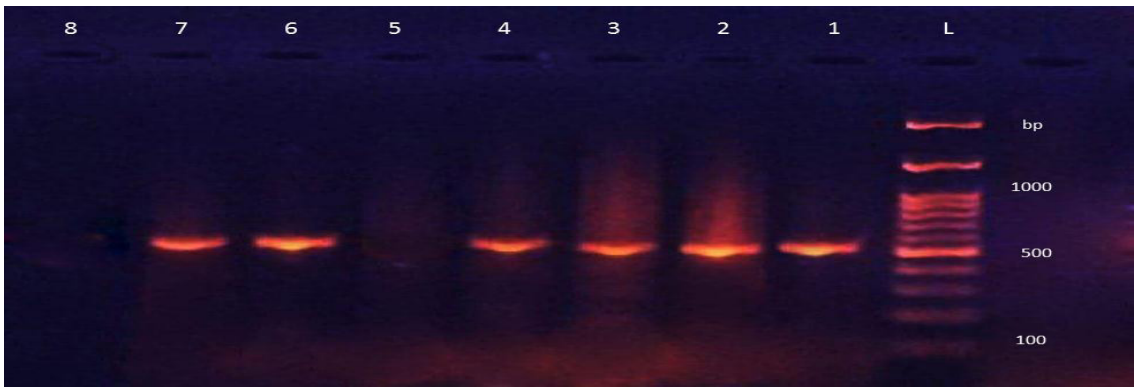


Figure (2): PCR amplification products of *Burkholderia cepacia* isolates that amplified with *blaCTX-M* gene primers with product 554bp.Lane (L), DNA molecular size marker (100-bp ladder), Lanes (1,2,3,4,6,7) show positive results with *blaCTX-M* gene.

ESBLs are one of the main leading causes of resistance to ß-lactam antibiotics among Gram-negative bacteria . These enzymes are plasmid-encoded β-lactamases that mediate resistance to penicillins, first-, second- and third- generation cephalosporins, such as cefotaxime, ceftriaxone, and ceftazidime . TEM, SHV, and CTX-M are the major genetic groups of ESBLs amongst clinically important Gram-negative bacteria . These enzymes are most commonly found in Klebsiella pneumoniae (K. pneumoniae) and Escherichia coli (E. coli) and are also observed in other clinical isolates of Enterobacteriaceae and Pseudomonas [15]. The first TEM-type β-lactamase, produced by a clinical E. coli strain, was reported in 1965. The TEM-type ESBLs are derivatives of TEM-1 and TEM-2. The SHV-type ESBLs may be found in clinical isolates more frequently than any other types of ESBLs and have been reported from several countries in Europe, such as Austria, France, Italy, and Greece, as well as in the United States and Australia . The CTX-M-type ESBLs developed from TEM and SHV and can be divided into five subgroups according to their amino acid sequence simi-larities, including CTX-M-I, CTX-M-II, CTX-M-III, CTX-M-IV, and CTX-M-V [16]

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

References

1- **Kirketerp**-Møller K, Jensen PØ, Fazli M, Madsen KG, Pedersen J, Moser C, Tolker-Nielsen T, Høiby N, Givskov M, Bjarnsholt T. *Distribution, organization, and ecology of bacteria in chronic wounds.* Journal of clinical microbiology.; (2008). 46; 8; p2717-22.

2- **DanceD**. Treatment and prophylaxis of melioidosis. Intj Antimicrob Agents. (2014).43:310-318.

3- **Spichler**, A.; Hurwitz, Bl.; Armstrong, D. And Lipsky, B. A. Microbiology Of Diabetic Foot Infections: From Louis Pasteur To 'Crime Scene Investigation'. Bmc Medicine. (2015).

4- **Wang,** Ah.; Xu, Zr. And Ji, Ln. Clinical Characteristics And Medical Costs Of Diabetics With Amputation At Central Kirketerp-Møller Urban Hospitals In China. Zhonghua Yi Xue Za Zhi; (2012). 92:224-7.

5- **Chessbrough**, M. District Laboratory Practice in Tropical Countries, Part-2, New York, USA, Cambridge University, (2010). 184- 186.

6- **Levy,** H.; Diallo, S.; Tennant, SM.; Livio, S.; Sow ,SO.; Tapia, M.; Fields, PI.; Mikoleit, M.; Tamboura, B.; Kotloff ,KL.; Lagos, R.; Nataro, JP.; Galen, JE.and Levine, MM. PCR Method to Identify *Salmonella Enterica Serovars Typhi*, *Paratyphi A*, and *Paratyphi B* Among *Salmonella* Isolates from the Blood of Patients with Clinical Enteric Fever. J Clin Microbiol, (2008). 46(5):1861-1866.

7- **Saladin**,M.;Cao,V.T.;Lambert,T.;Donay,J.L.;He rrmann,J.L.;Ould-Hocine,Z.;Verdat,C.;Delisle,F .;Philippon,A.andArlet ,G. Diversity of CTX-M beta- lactamases and their promoter regions from Enterobacteriaceae isolated in three Parisian hospitals. FEMS Microbiology Letters , (2002). 209:161-168.

8- **Bradford**,P.A.;Bratus,s.;Urban,C.;Visalli,M. andMariano,N. Emergence of Carbapenem-Resistant Klebsiella Species possessing the class A Carbapenem-Hydrolyzing KPC-2 and Inhibitor – Resistant TEM30 β -lactamases in New York City. Clin. infectious Dis ., (2004). 39:55-60.

9- **Dallenne,**C.,Dacosta,A.;Decre,D.;Favier,C. andArlet,G. Development of a set of Multiplex PCR assays for the detection of genes encoding important β –lactamases in Enterobacteriaceae .J.ANTImicrob.Chemother., (2010). 65:490-495.

10- **Antunes** NT, Lamoureaux TL, Toth M, Stewart NK, Frase H, Vakulenko SB. Class D ??-Lactamases: Are They All Carbapenemases? Antimicrob Agents Chemother. (2014). 58(4):2119–25.

11- **Manenzhe** RIZH, Nicol MP, Kaba M. The spread of carbapenemase producing bacteria in Africa: a systematic review. J Antimicrob Chemother. (2015).70(1):23–40.

12- **Walsh**, T. R., M. A. Toleman, L. Poirel, and P. Nordmann. Metallo- -lactamases: the quiet before the storm? Clin. Microbiol. Rev.)2005(. 18:306–325.

13- **Nagaraj** S, Chandran SP, Shamanna P, Macaden R. Carbapenem resistance among Escherichia coli and Klebsiella pneumoniae in a tertiary care hospital in south India. Indian J. Med. Microbiol. (2012) . 30(1): 93-95.

14- **Francis RO**, Wu F, Phyllis DL, Shi J and Whittier S. Rapid Detection of Klebsiella pneumoniae Carbapenemase Genes in Enterobacteriaceae Directly From Blood Culture Bottles by Real-Time PCR. Am J Clin Pathol. (2012). 137(4):627-32.

15- **Adeyankinnu** FA, Motayo BO, Akinduti A, Akinbo J, Ogiogwa JI, Aboderin BW, Agunlejika RA. A multicenter study of betalactamase resistant Escherichia coli and Klebsiella pneumoniae reveals high level chromosome mediated extended spectrum β-lactamase resistance in Ogun state, Nigeria. (2014).

16- **Roschanski N**, Fischer J, Guerra B, Roesler U. Development of a multiplex real-time PCR for the rapid detection of the predominant beta-lactamase genes CTX-M, SHV, TEM and CITtype AmpCs in Enterobacteriaceae. PLoS One (2014). 9:e100956.

Evaluation the Antimicrobial Effect of Glycerin Magnesia on Some Bacteria, in-Vitro Study

Maher Saber Owain[1], Khalid Ismael Oleiwi[2], Ali Ghazi Atiyah[1], Mustafa Salah Hasan[1]

[1]Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, University of Tikrit/ Iraq.

Abstract

This study was aimed to assess the efficacy of glycerin magnesia on some bacteria. A thirty percent of glycerin magnesia were prepared as explained below. Many types of bacteria including *Proteus spp., Staphylococcus aureus, Staphylococcus epidermidis, Acinetobacter, E. coli, Pseudomonas aeruginosa, Salmonella spp.* were selected for this study and obtained from university of Tikrit, college of veterinary medicine. A bacterial broth were prepared, a then a sterile swab were emulsify in these broth and streaked on muller hinton agar plate and allowed till dry, then a holes were filled with a given glycerin magnesia and incubated for 24hrs. at 37℃ . The results showed that a higher antibacterial effects of glycerin magnesia against *Staphylococcus aureus* followed by *Proteus spp., Pseudomonas aeruginosa, Salmonella spp., Acinitobacter, E. coli, Staphylococcus epidermidis* respectively.

In conclusion, the glycerin magnesia have a wide range antibacterial effect and can be used in future in wound healing.

Key words: *glycerin magnesia, bacteria, in-vitro.*

Introduction

Magnesium (Mg) is an important ion found in the body human and animals, and may be observed as a drug with numerous clinical uses [(1)]. The total magnesium in human and animal body were 53 % stores in the bones, 27 % in muscle, 19 % in other soft tissues, about 0.5 % in the RBCs and about 0.3 % in the serum [(2)]. About half of Magnesium are existing as free and doesn't bound with albumin or other ion [(3)].

Magnesium sulfate (MgSO4) is a solid, odorless material that present as crystal powders or as colorless crystals needle like or as a crystalline powder with white color [(4)].

Magnesium sulfate have a wide uses in building, industrial/processing, agriculture, special care products, medicine, food processing [(5)].

Corresponding author:
Mustafa Salah Hasan
drmustafasalah7@gmail.com

Magnesium sulfate uses in human medicine including as an anticonvulsant for controlling and in preventing seizures especially in children who suffer from nephritis, lowering blood pressure in pregnant females who suffer from preeclampsia, Asthma attacks can also be treated with MgSO4. MgSO4 used in human and animals as a laxative and also it can also use in relieving cases of constipation [(6)].

Ismail & Shaker [(7)] reported that MgSO4 with glycerin have an antibacterial effects when used on wound. The addition of glycerin as a vehicle for MgSO4, some reported that glycerin increase the antimicrobial activity of material that emulsify in it [(8)].

The most pathogenic bacteria for human and animals were reported including Salmonella [(9-13)], E. coli [(14-17)], Staphylococcus spp. [(18)], Proteus spp. [(19)].

The current study aimed to assess the efficacy of glycerin magnesia against some bacteria.

Materials and Method

Glycerin Magnesia (30%): The mixture was

prepared by dissolved a 30 g of MgSO4 in 30 ml heated distilled water and mixing thoroughly. After a complete dissolving of MgSO4, the glycerin was added slowly to the mixture till a final volume of 100ml and using heat and continual stirring.

Many types of bacteria including *Proteus spp., Staphylococcus aureus, Staphylococcus epidermidis, Acinetobacter, E. coli, Pseudomonas aeruginosa*, *Salmonella spp.* were selected for this study and obtained from university of Tikrit, college of veterinary medicine. Each type of these bacteria were inoculated in 5 ml nutrient broth and incubated for 24hrs., then a sterile swab were emulsify in these broth and streaked on muller hinton agar plate with a central hole (which made in each of the plate with a sterile 2.0 mm diameter cork borers) and allowed till dry, then a holes were filled with a given glycerin magnesia and incubated for 24hrs. at 37C .

Results and Discussion

The current results obtained were showed a higher antibacterial effects of glycerin magnesia against *Staphylococcus aureus* followed by *proteus spp*., *Pseudomonas aeruginosa*, *Salmonella spp., Acinitobacter*, *E. coli, Staphylococcus epidermidis* respectively (Table 1) (figures 1, 2).

Table (1) Antibacterial effects of glycerin magnesia on some bacteria.

Type of Bacteria	Diameter in mm
Staphylococcus aureus	40
Staphylococcus epidermidis	5
E. coli	25
Pseudomonas aeruginosa	30
Acinitobacter	28
Proteus spp.	35
Salmonella spp.	30

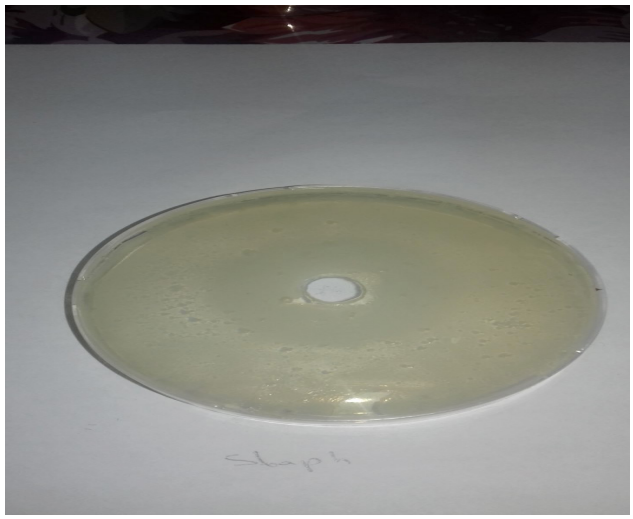


Figure 1 results of *S. aureus*

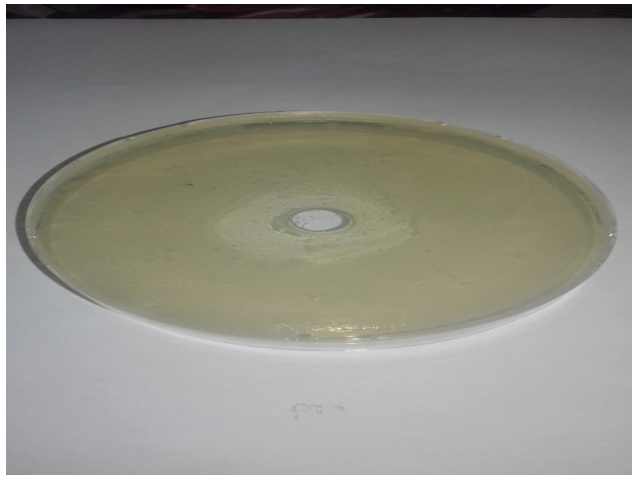


Figure 2 results of *E. coli*

It has been reported that Mg alone has an antibacterial effect invitro by reduction the bacterial growth and prevent the formation of biofilm by increasing of local alkalinity [20]. Robinson *et al*. [21] suggested that the alkaline pH was liable for the significance decreasing of CFU/ml[1].

Another researcher reported that the pH and the graded charge were significant in prokaryotic physiology in production the proton motion force that used to do the useful work for bacteria, also reported that a pH is important for extra and intracellular environments favored for growth of organisms [22]. Also, other researcher reported that some biomaterial have an ability to yield the alkaline pH which give rise the antibacterial mechanism [23, 24].

Li *et al*. [20] reported that pure magnesium when added to medium has a full effect on MRSA when tested in-vitro.

Also, Crisler *et al*. [25] found that the increasing medium salinity to 10% MgSO4 reduced the number of *Pseudomonas*. However, a report of Marnocha *et al*. [26] suggested that MgSO4 at 10% reduce the growth of most tested bacteria.

Many studies have been suggested that there was an association amongst the cell membrane fluidity and the stress tolerance. The structure of cell membrane fatty acids is liable for preservation of cell membrane fluidity. The most consequences of altering in fatty acid of cell membrane in most microorganisms are inflection the action of cells intrinsic proteins which achieve many purposes for example uptake of nutrient and ion pumping [27]. The treatment by using alkaline material resulted in alterations in fatty acid composition of the cell membrane for some bacteria especially *E. coli* and *Salmonella Spp*. which leading to reduction in number of these bacterial types [28].

Conclusion

Glycerin magnesia have a wide range antibacterial effect on many bacteria especially *S. aureus*, *Pseudomonas* and other, the future study will use this preparation in wound healing.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

References

1. Wacker WE & Parisi A F.. Magnesium metabolism. New England Journal of Medicine, 1968; 278(13): 712-717.
2. Facchinetti F, Sances G, Borella P, Genazzani A & Nappi G. Magnesium prophylaxis of menstrual migraine: effects on intracellular magnesium. *Headache:The Journal of Head and Face Pain*, 1991; 31(5): 298-301.
3. Jahnen-Dechent W & Ketteler M. Magnesium basics. *Clinical kidney journal*, 2012; 5(Suppl_1): 3-14.
4. Kawamura Y & Rao M. Magnesium Sulfate: Chemical and Technical Assessment, 2007.
5. Giles Chemical. Typical Uses of Magnesium Sulfate, dated October 27, 2008. Retrieved December 9, 2010, from http://www.gileschemical.com/pdfs/MGSO4TYP.pdf.
6. Adnani A, Basri M, Malek E A, Salleh A B, Rahman M B A, Chaibakhsh N & Rahman R N. Optimization of lipase-catalyzed synthesis of xylitol ester by Taguchi robust design method. *Industrial Crops and Products*, 2010; 31(2): 350-356.
7. Ismail T & Shaker O. Evaluation of the Potentials of Autologous Blood Injection for Healing in Diabetic Foot Ulcers. *Journal of the American College of Clinical Wound Specialists*, 2012; 4(2): 45-50.
8. Nalawade T M, Bhat K & Sogi SH. Bactericidal activity of propylene glycol, glycerine, polyethylene glycol 400, and polyethylene glycol 1000 against selected microorganisms. *Journal of International Society of Preventive & Community Dentistry*, 2015; 5(2): 114.
9. Hasan M S, Abdulgafor A B, Hussein M A, Owain M S, Aboud Q M & Al-Zobaie A J. In-vitro and in-vivo therapeutic study the effect of Ca-EDTA against Salmonella typhimurium infection in experimental local rabbits. *Journal of Pharmaceutical Sciences and Research*, 2018; 10(7): 1765-1767.
10. Hasan M S, Hussein M, Owain M and Yousif A. Clinical signs following experimental Salmonella typhimurium infection in rabbits. Online Journal of Veterinary Research, 2018; 22 (5):369-373.
11. Jarad A S, Ibrahim A Y, Ali R K, Hasan M S, Owain, M S and Hussein M A. Effect of gentamycin and calcium EDTA on mesenteric lymph nodes in rabbits following oral *Salmonella typhimurium* infection. Online Journal of Veterinary Research, 2018; 22 (7):596-601.
12. Abdulgafor A B, Owain M S, Hasan M S, Hussein M A, Aboud Q M & Al-Zobaie A J. Liver, kidney function tests and oxidative damage during and after treatment of Salmonella typhimurium infection in experimental local rabbits. *International Journal of Pharmaceutical Quality Assurance*, 2018; *9*:5-6.
13. Hussein M A, Owain M S, Abdulgafor A B, Hasan M S, Jarad A S, Aboud Q. M & Al-Zobaie A J. Study the Pathological Changes in the Intestine of Rabbits Infected Experimentally with *Salmonella typhimurium*. Inter J Vet Sci., 2019; 8(2): 79-83.
14. Hasan M S, Yousif A A & Alwan M J. Detection of virulent genes in E. coli O157: H7 isolated from puppies and adult dogs by polymerase chain reaction. *Res. J. Vet. Pract*, 2016; *4*(1): 1-6.

15. Hasan M S, Hussein M A & Yousif A A. Confirmatory detection of Escherichia coli O157: H7 in diarrheic and non-diarrheic calves by using real time PCR with studying the antimicrobial susceptibility of these bacteria. *Journal of global pharma technology*, 2018; *10*:9-10.
16. Yousif A A, Hasan M S, Najim T M and Hussein M A. Detection of virulence and antimicrobial susceptibility testing of Escherichia coli O157:H7 isolated from pets and stray dogs. Journal of Entomology and Zoology Studies, 2017; 5(4): 573-578.
17. Hassan M S and Yousif A A and Mohammad J Alwan. Clinical and molecular study of *E. coli* O157:H7 isolated from Diarrheic and non-diarrheic dogs. MRVSA, 2016; 5 (2): 1-10.
18. Hassan M S and Yousif A A . Alteration of some enzymatic activities in whey of ewe's milk Suffered from Staphylococcal mastitis Mirror of Research in Veterinary Sciences and animals. MRSVA, 2013; 2 (2): 8-15.
19. Hassan M S and Yousif A A. Evaluation of some biochemical indices in "milk whey" for diagnosis of subclinical mastitis caused by some *Enterobacteriaceae* family. Mirror of Research in Veterinary Sciences and animals. MRSVA, 2014; 3 (1): 1-7.
20. Li Y, Liu G, Zhai Z, Liu L, Li H, Yang K & Yu Z. Antibacterial properties of magnesium in vitro and in an in vivo model of implant-associated methicillin-resistant Staphylococcus aureus infection. *Antimicrobial agents and chemotherapy*, 2014; *58*(12): 7586-7591.
21. Robinson D A, Griffith R W, Shechtman D, Evans R B & Conzemius M G. In vitro antibacterial properties of magnesium metal against Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus. *Acta biomaterialia*, 2010; *6*(5): 1869-1877.
22. White D. Membrane bioenergetics: the proton potential. In: The physiology and biochemistry of prokaryotes, 3rd ed. New York: Oxford University Press, 2007: 83–119.
23. Stoor P, Söderling E & Salonen J I. Antibacterial effects of a bioactive glass paste on oral microorganisms. *Acta Odontologica Scandinavica*, 1998; *56*(3): 161-165.
24. Hu S, Chang J, Liu M & Ning C. Study on antibacterial effect of 45S5 Bioglass®. *Journal of Materials Science: Materials in Medicine*, 2009; *20*(1): 281-286.
25. Crisler J D, Newville T M, Chen F, Clark B C & Schneegurt M A. Bacterial growth at the high concentrations of magnesium sulfate found in Martian soils. *Astrobiology*, 2012; *12*(2): 98-106.
26. Marnocha C L, Chevrier V F & Ivey D M. Growth of sulfate-reducing bacteria in sulfate brines and the astrobiological implications for Mars. In *Lunar and Planetary Science Conference*, 2011; 42: 1604).
27. Russell N J & Fukunaga N. A comparison of thermal adaptation of membrane lipids in psychrophilic and thermophilic bacteria. *FEMS Microbiology Reviews*, 1990; *6*(2-3): 171-182.
28. Sampathkumar B, Khachatourians G G & Korber D R. Treatment of Salmonella enterica serovar Enteritidis with a sublethal concentration of trisodium phosphate or alkaline pH induces thermotolerance. *Appl. Environ. Microbiol.*, 2004; *70*(8): 4613-4620.

The Role of CTX-II , Dyslipidemia ,Vitamin D in Polycystic Ovary Syndrome

Samah Amer Hammood[1] Alaauldeen S. M. AL-Sallami[2]

[1]Lecturer: Laboratory Investigation department / Science Faculty / Kufa University, Iraq, [2]Prof: Department of Biology, Science Faculty / Kufa University, Iraq

Abstract

This study aimed to study the serum measurement of clinical value of type II collagen (CTX-II) in syndrome of polycystic ovary in women and indicter as osteoarthritis disease marker analyzed in future levels with a recent available immune enzyme-linked sorbent assay (ELISA) kit. For increasing sensitivity test, the protocol was modified. Levels of CTX-II increased significantly as well total protein, while decrease vitamin D and difference in lipid profile were practical between the patients compared with healthy women's. The obtained results suggested the monitoring interest of the serum CTX-II for the OA development in patients of polycystic ovary syndrome in women and the relevance of the analysis of multiple time point for this biomarker.

Keyword: *CTX-II- Biomarkers- OA Osteoarthritis- Polycystic ovary syndrome- Dyslipidemia.*

Introduction

Syndrome of polycystic ovary (PCOS) is a hard condition with characteristics of prominent levels of androgen, irregularities of menstrual and/or small cysts on either ovaries or both [(1)]. This disorder might be of ovaries morphological polycystic or hyper androgenemia predominantly biochemical. Hyper androgenism, is a PCOS clinical hallmark result of inhibition of follicular progression, ovaries microcysts, , changes in menstrual and ovulation [(2)]. Investigations proposed that (5% to 10%) females of age 18 - 44 years are PCOS affected, rendering it the most well-known abnormality of endocrine among females of reproductive age in U.S.A. [(3)]. Women looking for assistance from professionals in health care to address issues of excessive hair growth, obesity, acne, infertility, and amenorrhea most of the times receive a PCOS diagnosis and have cancer of endometrial at higher rates, TIIDM, dyslipidemia and cardiovascular disease [(4)].

PCOS pathophysiology involves defects primary in insulin secretion and action, ovarian function, and the hypothalamic–pituitary axis. Although the unknown cause of PCOS, PCOS has been connected to obesity and insulin resistance [(5)].There are 3 most well-known elements with PCOS associated i.e. irregularities of ovulation, androgen levels enhancement, and problems of cystic ovaries with elevated androgen levels and ovulation take place in most PCOS women [(24, 26)]. Furthermore, alopecia, hirsutism, and acne are directly associated with elevation levels of androgen and the ovaries prevalence of polycystic on pelvic ultrasound more than 70% in PCOS patients [(6)].

After diagnosing PCOS, investigations prove that patients over 50% have diabetes or pre-diabetes, and there is risk increasing of hypertension, myocardial infarction (MI), dyslipidemia, osteoarthritis sleep apnea, anxiety, depression, and endometrial cancer. Furthermore, PCOS pregnant women should be noticed for increasing miscarriage rates, pre-eclampsia, premature delivery, and gestational diabetes [(7)]. Osteoarthritis defined as a disease with developing articulate cartilage destruction and by changes pathologically in the subchondral bone and synovial membrane · OA, the destruction will result in losing the 2 major components, type II collagen and proteoglycans, rendering them choice markers in determining metabolism of cartilage [(8)]. Peptide of C-telo of collagen type II (CTX-II) is marker most studied [(9)]. Increasing levels were documented in OA patients

Corresponding author:
samah.alobaidi@uokufa.edu.iq
Samah Amer Hammood

in comparison to subjects of asymptomatic or without OA signs [10]. A significant association have shown between CTXII concentration and OA radiographic development [11].The goal of this investigation was to study the serum measurement of clinical value of CTX-II in polycystic ovary syndrome patients and correlated with physiological assessment that give indicator to development of Osteoarthritis in future.

Methodology

Serum specimen was collected from patient with infertile Polycystic Ovary patients (n = 45) and healthy patient (n=45) at AL-Sader laboratory of Medical city in Najaf Province, AL-Najaf Health Directorate / Ministry of Health /Iraq. The average of the patient's age was (32.81±51) years. All reagents and specimens should be at a temperature of room before use. Reagents mixed in soft way with no foaming. No interruption should take place once the protocol started Tests of biochemical were performed at Biology laboratories. in this study was CTX-II protein (MBS2507692), vitamin D3 (MBS773966) , HDL (MBS170439) , VLDL(MBS265004), total protein (MBS2540455) and LDL (MBS162140) My Bio Source Company USA in Origin .

Statistical Analysis

Statistical analyses of all result were carried out by the help of Graphpad prism version 5) software statistical package using t-test (with p value at level of significant less than 0.05) to compare values of result between groups .

Results

The result show an increase of significant for protein of collagen matrix Type II figure(1) in patient with Polycystic Ovary(mean± Std. Error 3.98+0.67) compare control group (mean± Std. Error 2.62+0.71) which that may be cause by the extracellular adhesion molecule is soluble from the cartilage surface component. Enable the binding of cartilage cells to type II collagen in the absence of serum, thereby increasing protein externally, we conclude that direct interaction between cartilage cells and type II collagen occurs through other adhesion mechanisms of cell surface proteoglycans such as membrane-bound heparin sulfate in progressive age [12]. Few studies revealed a significant relation between concentration of CTXII and OA radiographic development [13]. Recently, urinary levels predictive value of CTX-II for cartilage losing was assessed through MRI [14].

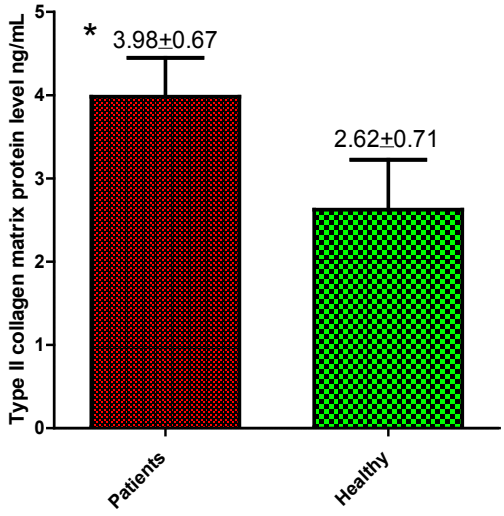


Figure 1: levels of Type II protein collagen matrix protein in patients comparison to Healthy group

These results was show a decrease of vitamin D3 in patient with Polycystic Ovary (mean± Std. Error 12.5±0.17) compare control group (mean± Std. Error 24.4±0.91) figure (2), Probably Vitamin D deficiency may be associated with populations with extensive skin coverage, especially in women in Iraq. There is a large body of proof the importance of vitamin D in reproductive function because VDRs have been detected in placenta, the endometrium and ovary [15]. Deficiency of Vitamin D is associated with deregulation of Ca, which participates in the follicular arrest development in PCOS women resulting in dysfunction fertility and menstrual [16]. Some study on PCOS women with and undergoing fertilization *in vitro* (IVF), they found that the women who achieved pregnancy exhibited significantly higher levels of follicular fluid of 25(OH) D and each ng/ml elevate in follicular fluid 25(OH)D elevated the likelihood for pregnancy achievement by 7% [17]. Moreover, deficiency of 25(OH)D was related with rates of lower development for pregnancy and follicle after clomiphene-citrate stimulation in PCOS women, suggesting a possible vitamin D supplementation role in PCOS infertile women who undergo stimulation of ovarian [18].

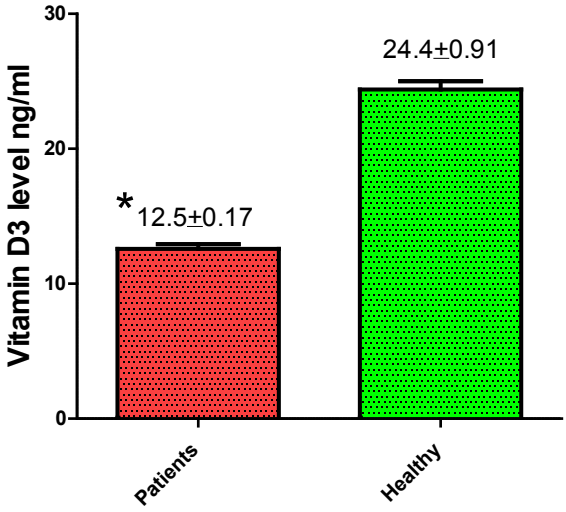


Figure 2: levels of vitamin D3 in patients comparison to Healthy group

These results was show a increase of total protein in patient with Polycystic Ovary (mean± Std. Error 8.01±1.34) compare control group (mean± Std. Error 6.65±0.98) figure (3), Probably by understanding the general proteins alterations not only of advantage for PCOS pathogenesis mechanism elucidating, but also easing for discovering the specific biomarkers and sensitive that are closely linked to diseases. Proteins quantitative analysis in serum with array protein provides useful data for clinical practicing i.e. individualized treatment and accurate medical that is according to protein screening. Five hundred proteins were examined by array of protein in samples of serum collected from rectal cancer where the OPG expression of was elevated which was associated with the patients survival of rectal cancer after chemotherapy [19]

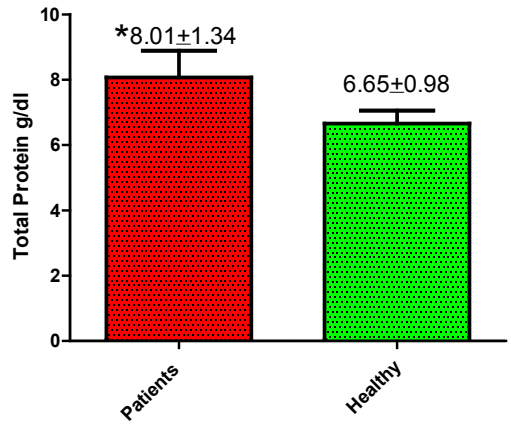


Figure 3: levels of total protein in patients comparison to standard group

The results revealed an increasing levels of significant in LDL level in patient with polycystic (mean± Std. Error 118±0.64) ; control group (mean± Std. Error 44.4±0.26) figure(4), also The result show decrease significant level in HDL level in patient polycystic (mean± Std. Error 39.4±1.14) ; control group (mean± Std. Error 49.4±2.41) figure (5),suggests that the level of LDL, HDL, insulin, glucose, apolipo protein A1, and lipoprotein a, in female with and without PCOS do not show any significant differences [20]. Similarly, Jahanfar *et al*., in a study aimed at evaluating the genetic and environmental factors affecting lipids among twins, found no significant difference [21] .The research concluded that the serum CTX-II in women with PCOS may appear to have symptoms of Osteoarthritis and thus it is considered to be a vital reliable evidence in the news of the occurrence of this disease in PCOS women.

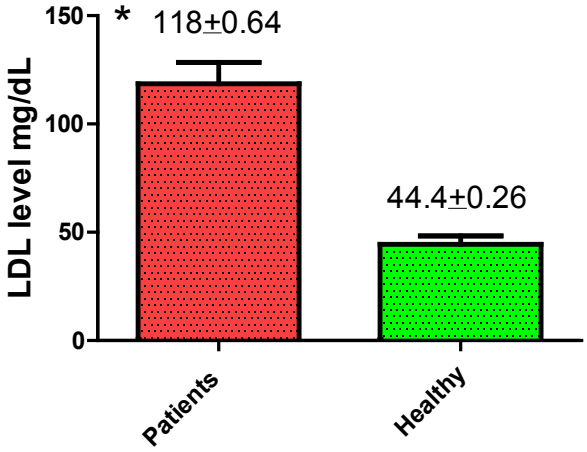


Figure 4: levels of LDL in patients comparison to Healthy group

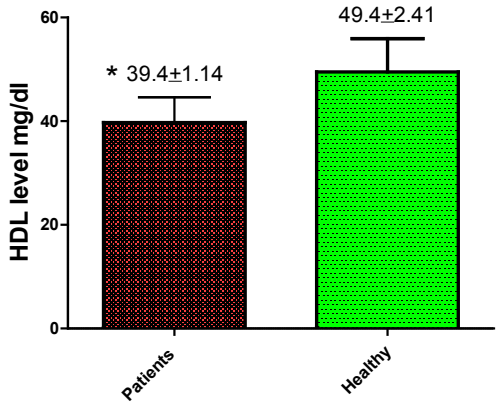


Figure 5: levels of HDL in patients comparison to Healthy group

Results proved a significant enhancement of VLDL level in patient (mean± Std. Error 29±2.34) ; control group (mean± Std. Error 20±2.64) figure(6) , Disturbances in metabolic are famous clinical syndrome characters, especially, dyslipidemia which is very well-known abnormality metabolic in PCOS female with a prevalence of up to 70% [22,23]. Resistance of insulin is a key PCOS pathophysiology and dyslipidemia in PCOS women may be therefore in accordance with that detected in the insulin resistant case: decreasing levels of apolipoprotein (Apo) A-I, and high-density lipoprotein-cholesterol (HDL-C), and increased levels of ApoB, triglycerides (TG) and very low-density lipoprotein[24, 25, 26].

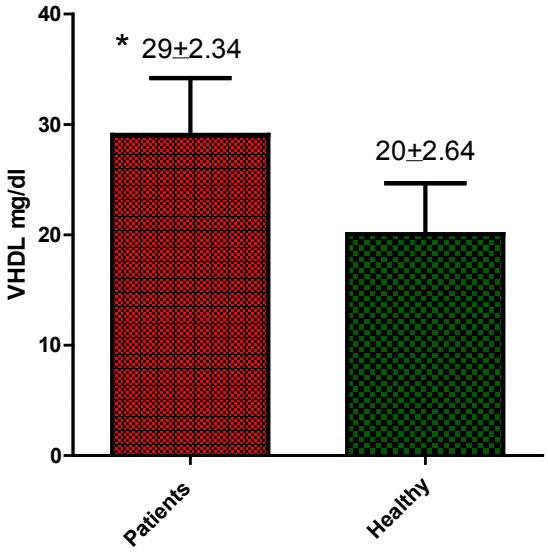


Figure 6: levels of VLDL in patients comparison to Healthy group

Conclusion

The research has a goal of examining the clinical value of the serum which was measured type II collagen (CTX-II) in the Polycystic ovary syndrome in the women and indicter as a marker of the (OA)disease of future levels. An important percentage increased in CTX-II levels , and the total protein but a decrease occurred in vitamin D and with a change in lipid profile which was in practical status between the patients compared with healthy women.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

References

1. Umland EM, Weinstein LC, Buchanan EM. Menstruation related disorders. *In:* DiPiro JT, Talbert RL, Yee GC, et al. *Pharmacotherapy: A Pathophysiologic Approach*, 8th ed. New York: McGraw-Hill; 2011:1393.
2. Lin LH, Baracat MC, Gustavo AR, et al. Androgen receptor gene polymorphism and polycystic ovary syndrome. *Int J GynaecolObstet* 2013;120:115–118.
3. National Institutes of Health, Department of Health and Human Services. *Beyond Infertility: Polycystic Ovary Syndrome (PCOS)*.NIH Pub. No. 08-5863, April 2008. Available at: www.nichd.nih.gov/publications/pubs/upload/PCOS_booklet.pdf. Accessed March 27, 2013.
4. McFarland C. Treating polycystic ovary syndrome and infertility.*MCN Am J Matern Child Nurs* 2012;37(2):116–121.
5. Shannon M, Wang Y. Polycystic ovary syndrome: A common but often unrecognized condition. *J Midwifery Womens Health*2012;57:221–230.
6. Azziz R, Carmina E, Dewailly D, et al. Position statement: Criteriafor defining polycystic ovary syndrome as a predominantly hyper androgenic syndrome. An Androgen Excess Society guideline .*J Clin Edocrinol Metab* 2006;91:4237–4245.
7. American Congress of Obstetricians and Gynecologists. ACOG Practice Bulletin No. 108: Polycystic Ovary Syndrome. *Obstet Gynecol* 2009;114(4):936–949.
8. Samuels J, Krasnokutsky S, Abramson SB. Osteoarthritis: a tale of three tissues. Bull NYU Hosp Jt Dis 2008;66:244e50.
9. Charni-Ben Tabassi N, Garnero P. Monitoring cartilage turnover. Curr Rheumatol Rep 2007;9:16e24.
10. Cibere J, Zhang H, Garnero P, Poole AR, Lobanok T, SaxneT,et al. Association of biomarkers with pre-radio graphically defined and radio graphically defined knee osteoarthritis in a population-based study. Arthritis Rheum 2009;60:1372e80.
11. Mazieres B, Garnero P, Gueguen A, Abbal M, BerdahL, Lequesne M, et al. Molecular markers

of cartilage break down and synov it is at baseline as predictors of structural progression of hip osteoarthritis. The ECHODIAH Cohort. Ann Rheum Dis 2006;65:354e9.

12. Jugdaohsingh R, Calomme MR, Robinson K, Nielsen F,Anderson SH, D'Haese P, et al. Increased longitudinal growth inrats on a silicon-depleted diet. Bone 2008;43:596e606.
13. Mazieres B, Garnero P, Gueguen A, Abbal M, BerdahL,Lequesne M, et al. Molecular markers of cartilage breakdown and synovitis at baseline as predictors of structural progression of hip osteoarthritis. The ECHODIAH Cohort. Ann Rheum Dis 2006;65:354e9.
14. Dam EB, Byrjalsen I, Karsdal MA, Q vist P, Christiansen C. Increased urinary excretion of C-telopeptides of type IIcollagen (CTX-II) predicts cartilage loss over 21 months byMRI. Osteoarthritis Cartilage 2009;17:384e9.
15. Sirmans SM, Pate KA. Epidemiology, diagnosis, and management of polycystic ovary syndrome. Clin Epidemiol. 2013;6:1-13.
16. Carrie C, Dennett, Judy Simon. The role of polycystic ovary syndrome in reproductive and metabolic health: overview and approaches for treatment. Diabetes Spectr. 2015;28(2):116-20.
17. Jukic AMZ, Upson K, Harmon QE, Baird DD. Increasing serum 25-hydroxyvitamin D is associated with reduced odds of long menstrual cycles in a cross-sectional study of African American women. Fertil Steril. 2016;106(1):172-9.
18. Butts SF, Seifer DB, Koelper N, Senapati S, Sammel MD, Hoofnagle AN, et al. Vitamin D Deficiency is Associated with Poor Ovarian Stimulation Outcome in PCOS but not Unexplained Infertility. J Clin Endocrinol Metab. 2018.
19. Meltzer S, Kalanxhi E, Hektoen HH, Dueland S, Flatmark K, Redalen KR, Ree AH. Systemic release of osteoprotegerin during oxaliplatin-containing induction chemotherapy and favorable systemic outcome of sequential radiotherapy in rectal cancer. Oncotarget 2016; 7: 34907-34917.
20. Bahceci M, Aydemir M, Tuzcu A. Effects of oral fat and glucose tolerance test on serum lipid profile, apolipoprotein, and CRP concentration, and insulin resistance in patients with polycystic ovary syndrome. Fertil Steril 2007; 87: 1363-8.
21. Jahanfar S, Eden JA, Wang XL, Wilcken DE, Nguyen T. The effect of genetical and environmental factors on lipids: A twin study'. Medical Journal of the Islamic Republic of Iran 1998; 12: 5-9.
22. Legro RS, Kunselman AR, Dunaif A. Prevalence and predictors of dyslipidemia in women with polycystic ovary syndrome. Am J Med. 2001;111:607–613.
23. Taskinen MR. LDL-cholesterol, HDL-cholesterol or triglycerides: which is the culprit? Diabetes Res Clin Pract. 2003;61(Suppl 1):S19–S26.
24- Al-Masaoodi, RusulAli, Al-Sallami, Alaauldeen S.M. and Al-Baseesee, Hamadallah. The Role of Inflammatory Markers in the Development of the Osteoporosis in Women after Menopausal. Indian Journal of Public Health Research & Development.2019;10(8):1068-1073.
25- Aljelawy,Zahraa H. and Al-Sallami, Alaauldeen S. M. Role of kisspeptin in polycystic ovary disease in patients of ALNajaf ALAshrif City, Iraq. J. Pharm. Sci. & Res.2019 Vol. 11(1), 169-173.
26- Al-Masaoodi, Rusul Ali, Al-Sallami, Alaauldeen S.M. and Al-Baseesee, Hamadallah. The relation between the RANKL and resistin in menopausal women with osteoporosis. AIP Conference Proceedings.2019.Volume2144,Issue1. https://doi.org/10.1063/1.5123113

Prevalence of Histopathological Diagnosis of Benign and Malignant Breast Lesions in Al-Muthanna Province for Two Years Duration

Sarah Ali Abed[1]; Ali Adnan Jabbar[2]; Roaa Salih Mahdi[3]; Ali Mohammed Khaleel[4]; Suha Suhiel Hussein[5]; Maha Khadum Ghayadh[6]

[1]Lecturer, College of Medicine, Al-muthanna University/Iraq, [2] Ass. Prof. Dr. College of Medicine, Al-muthanna University/Iraq, [3]Lecturer, College of Medicine, University of Babylon/Iraq, [4]Dr. Specialist F.I.B.M.S., Babylon health director /MOH/Iraq, [5]Dr. Specialist M.SC., Al- Muthanna health director/MOH/Iraq, [6]Dr. MD (MPATH, USM, MALASIA), Al- Muthanna Health Director/MOH/Iraq

Abstract

Introduction: Breast cancer (BC) is a common form of cancer among women globally. It is considered 5th cause of death in females with equally to 522,000 patients / year. Nowadays it is considered 15.4% of death in developed countries after lung cancer. In developing nations, breast cancer considered the 1st cause of death for females 324,000 cases of deaths (14.3%) for total deaths. **Method:** One hundred and six female patients with breast lesion were collected from the Al-Hussein Teaching Hospital Laboratory/Histopathology department –Al-Muthanna province, during the period from January 2018 to January 2020. The data for cases were collected to study the age, type of breast lesion whether benign or malignant. Haematoxylin/ Eosin staining done in Al-Hussein Teaching Hospital Laboratory / Histopathology department. **Results:** Cross sectional study for 106 patients done in Al- Muthanna proven for assessment breast mass and identify malignant and benign lesion, mean age of patients was (39.4 ± 15.8) years old, with min age 13 years and max age was 81 years old. Types of biopsy taken from surgeon were 71% excisional biopsy, 21% mastectomy and 8% true cut biopsy. After pathological assessment of biopsies showed 51% malignant and 49% benign. In addition, this assessment distributed as following: fibroadenoma 31.1%, IDC/ grade II 29.2%, IDC/ grade III 9.4%, ILC and fibrocystic changes 5.7% and IDC grade I 3.8% and other types of malignant and benign after pathological assessment. Significant association between age groups and cancer of breast. **Conclusion:** After pathological assessment of biopsies, malignant breast cancer (IDC/ grade II, IDC/ grade III, ILC and fibrocystic changes) most common changes, significant association between age group and breast cancer development more age group 41- 50 years old and then (31- 40), (51– 60) years old respectively.

Key words: *Prevalence, histopathological diagnosis, breast lesions, Al-muthanna province*

Introduction

Breast cancer (BC) is a common form of cancer among women globally. It is considered 5th cause of death in females with equally to 522,000 patients / year [(1)]. Nowadays it is considered 15.4% of death in developed countries after lung cancer [(2)]. In developing nations, breast cancer considered the 1st cause of death for females 324,000 cases of deaths (14.3%) for total deaths [(2, 3)]. This rate changes from 6 – 20 / 100.000 in West Africa and East Asia (4). The incidence in 2012 in females reach to 1.7 million (25% of entirely cancers), 883,000 patients in developed country in contradiction of 794,000 in developing nations [(4, 5)].

The incidence was increase in age after 35 years old and peaking in 60 years old [(6)].

Mortality rate depend on age of females, staging of malignancy, treatment respond, metastasis of malignancy, main reasons of breast cancer is hormonal factors, genetic tendencies, behaviors and ecological reasons [(7)].

Corresponding author:
Sarah Ali Abed
qaisajam1981@gmail.com

Benign Breast Diseases (BBDs) is not cancer. Most usual reason of breast difficulty in women and more usual than malignancy (8, 9). It is more usual than malignancy in western countries (10). More than 30% of women with BBD need treatment where survive (11). A three-ways for evaluation done by clinical assessment: US and mammogram, pathological assessment, central needle biopsy. Most women with BBD not have risk of development of breast cancer, so early diagnosis and treatment is important to remove the anxiety especially women with family history of breast cancer and must follow up annually. Most common BBD is fibro adenoma (12). Two types of breast cancer (cancer in situ): **Ductal carcinoma in situ. (**DCIS) 83% of all cases 2010-2014 unusual cells substitute usual epithelial cells around ducts of breast and metastasis to the lobules in addition to the ducts; it is can or can not developed to the aggressive malignancy it is grow slowly without any management it is misdiagnosis with benign 20%-53% diagnosed with an invasive breast malignance for 10 years or more. **Lobular carcinoma in situ.**: 13 % of patients, atypical cells developing inside and growing about some of breast lobules. It is not precursor of invasive cancer, but considered one of strong cause of invasive cancer. The aim of study is to show proportion of benign and malignant breast lesions and types of malignancy tissues by pathological assessment and relation of malignancy with increase age of females (13-15).

Method

One hundred and six female patients with breast lesion were collected from the Al-Hussein Teaching Hospital Laboratory/Histopathology department –Al-Muthanna province, during the period from January 2018 to January 2020. The data for cases were collected to study the age, type of breast lesion whether benign or malignant. Haematoxylin/Eosin staining done in Al-Hussein Teaching Hospital Laboratory / Histopathology department.

***Specimens**:*

The one hundred and six cases classified into benign and malignant breast lesions. From each formalin fixed paraffin embedded tissue, one section of 5-micron thickness was obtained and stained by haematoxylin and eosin staining method for evaluation of morphology.

Methods of staining procedures:

The following steps were applied for (H&E) staining method.

a) Deparaffinization: This done by adding the following:

1. 5 min. period adding Xylene.

2. 5 min. period adding Xylene.

3. 5 min. period adding 99 % ethanol

4. 5 min. period adding 99 % ethanol

5. 5 min. period adding 99 % ethanol

6. 5 min. period adding 99 % ethanol

7. 5 min. period adding 99 % ethanol

8. 5 min. period adding 95 % ethanol

9. 5 min. period adding 70 % ethanol

10. Purified water.

b) Hematoxyline and eosin staining method:

1. Dewax sections (deparaffinization as above).

2. Stain in hematoxyline for 3-10 minutes.

3. Wash well in running tap water.

4. Remove excess stain by differentiating the sections in 1% acid alcohol (1% in HCL 70% alcohol) for 5-10 seconds.

5. Wash well with in tap water until sections regain their blue color.

6. Stain in eosin for 2-5 minutes.

7. Dehydrate slowly through increasing grades of alcohol (i.e.70%, 90% and 100%).

8. Clearing by xylene.

9. Mount wit DPX.

Statistical analysis done by SPSS 22 calculated mean and SD with percentage and frequency. Chi square use for revealed association between age groups and behavior of tumor, significant association when P-value less than 0.05.

Results

Cross sectional study for 106 patients done in Al-

Muthanna proven for assessment breast mass and identify malignant and benign lesion, mean age of patients was (39.4 ± 15.8) years old, with min age 13 years and max age was 81 years old. Types of biopsy taken from surgeon were 71% excisional biopsy, 21% mastectomy and 8% true cut biopsy as showed in fig (1). After pathological assessment of biopsies showed 51% malignant and 49% benign as in fig (2). In addition, this assessment distributed as following: fibroadenoma 31.1%, IDC/ grade II 29.2%, IDC/ grade III 9.4%, ILC and fibrocystic changes 5.7% and IDC grade I 3.8% and other types of malignant and benign after pathological assessment showed in fig (3).

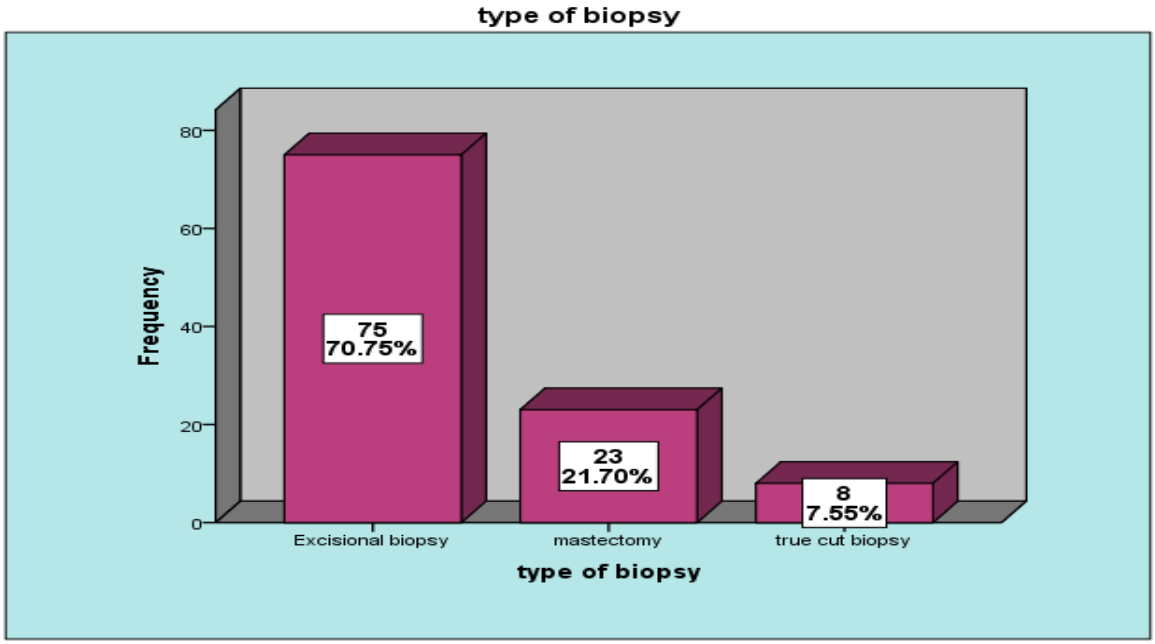


Fig (1): distribution of types of biopsy.

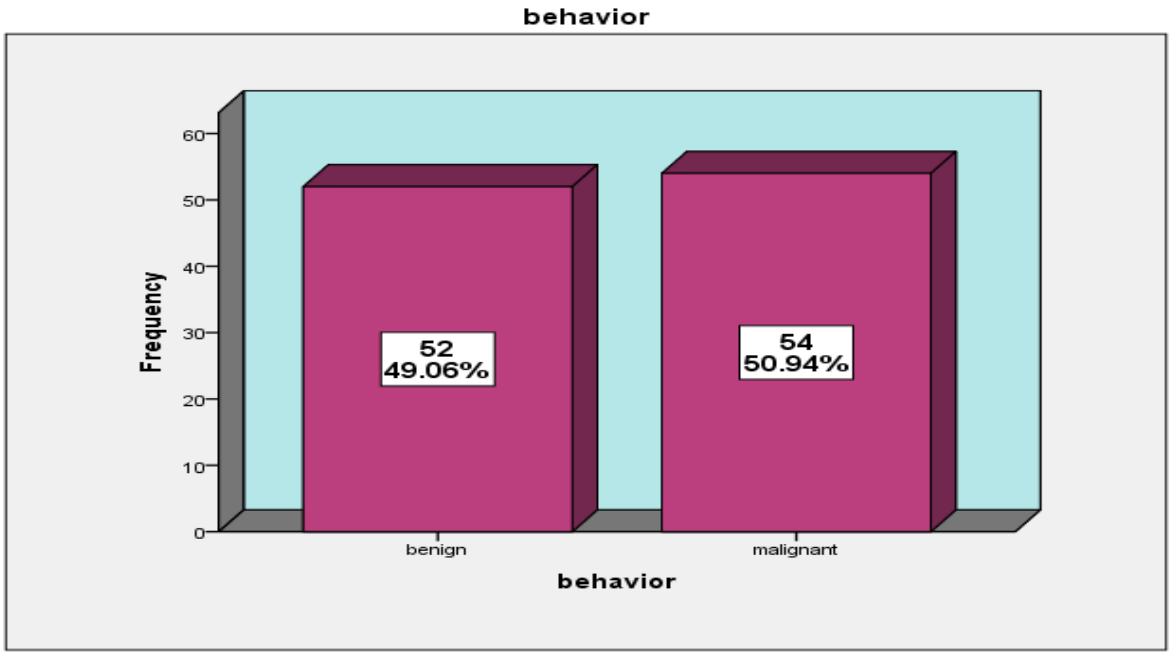


Fig (2): distribution of behavior of biopsy after pathological assessment.

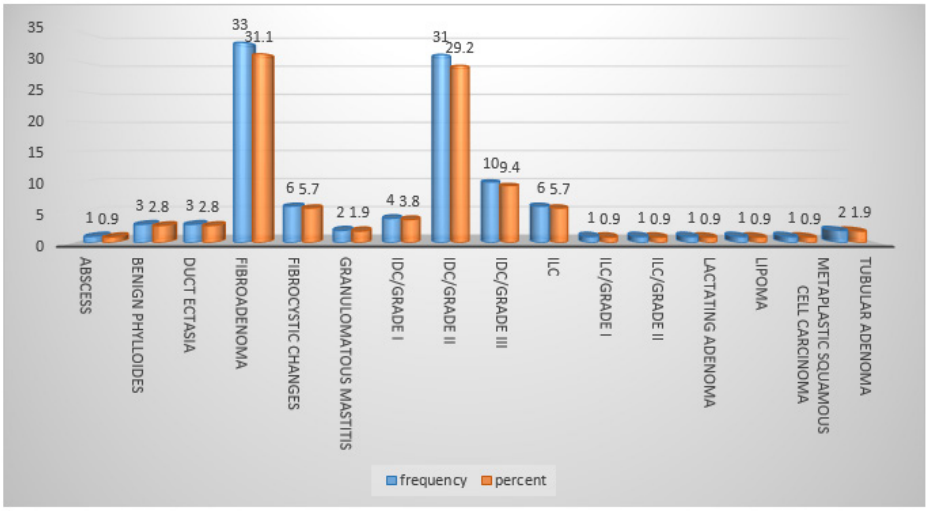


Fig (3): distribution of benign and malignant according to types after pathological assessment.

According to association between ages groups and behavior of lesion either benign or malignant, there is significant association between age groups and cancer of breast, 37% of females in age group 41- 50 years old with malignant breast cancer, while 20% of malignancy in age group (31 – 40) and (51 – 60) years old. 13% of malignancy in age group 61 – 70 years old and only 6% in female over 71 years old age. As show in table (1).

Table (1): association between age groups and behavior of lesion either benign or malignant.

benign malignant			**behavior**		**Total**
Age	11 - 20 years	Count	13	0	13
		% within behavior	25.0%	0.0%	12.3%
	21 - 30 years	Count	22	2	24
		% within behavior	42.3%	3.7%	22.6%
	31 - 40 years	Count	11	11	22
		% within behavior	21.2%	20.4%	20.8%
	41 - 50 years	Count	6	20	26
		% within behavior	11.5%	37.0%	24.5%
	51 - 60 years	Count	0	11	11
		% within behavior	0.0%	20.4%	10.4%
	61 - 70 years	Count	0	7	7
		% within behavior	0.0%	13.0%	6.6%
	more than 71 years	Count	0	3	3
		% within behavior	0.0%	5.6%	2.8%
Total		Count	52	54	106
% within behavior		100.0%	100.0%	100.0%	

Pearson Chi-Square= 58.188, P-value = 0.0001 (significant).**

Fig (4): Invasive ductal carcinoma with Hematoxylin & Eosin staining (10X)

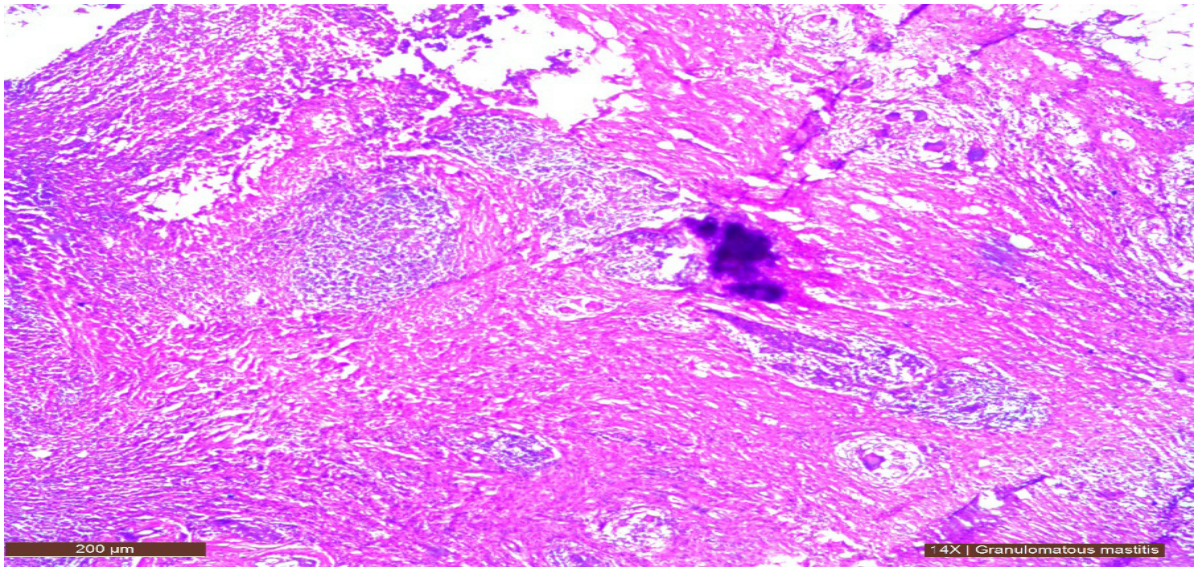


Fig (5): Granulomatous mastitis with Hematoxylin & Eosin staining (4X)

Discussion

Cancer is illnesses that lead the cells in body loss of control and change. All types of cancer cells finally changes to lump or mass that named cancer, and this malignant tumor called according to the site form it the tumor originate. Breast cancer start from gland of breast tissue that lead to production of milk called (terminal duct lobular unit). Other parts of breast tissue consist of connective, fatty and lymphatic tissues (16).

According to our study, mean age of patients was (39.4 ± 15.8) years old, with min. age 13 years and max. age was 81 years old. These results similar to study done by Augustin et al. showed the age extended since 16 - 90 years by a mean of 45.83± 13.5 years old (17). Zhi-Gang Yu et.al. Also, have the same results that mean age of patients was (44 ± 11.6) years old (18)characteristics and related factors of breast cancer among women in Eastern China. A total of 122,058 female subjects completed the study, with 320 confirmed cases of breast cancer (crude prevalence: 262.5/100,000; standardized prevalence: 207.7/100,000.

In our study the types of biopsy taken from surgeon were 71% excisional biopsy, 21% mastectomy and 8% true cut biopsy this is similar to Augustin et al. that showed, the total of cases that take and examine by pathologist contain of 69% of lumpectomy then 30% biopsy of breast.

After pathological assessment of biopsies in our study showed 51% malignant and 49% benign while in another study the proportion was around 4:1 or 5:1 (19), this different is due to sample collection or may be due to high no. of malignancy of breast in al-muthanna province.

In addition, this assessment distributed as following: fibroadenoma 31.1%, IDC/ grade II 29.2%, IDC/ grade III 9.4%, ILC and fibrocystic changes 5.7% and IDC grade I 3.8% and other types of malignant and benign after pathological assessment this results similar to Augustin et al. that showed he predominant tumor histological designs IDC 113 (64.9%), ILC 17 (9.8%) then histological designs were invasive ductal carcinoma 113 (64.9%), invasive lobular carcinoma 17 ductal carcinoma in situ 10 (5.7%) and medullary ca. 5 (2.8%) (17). Similar features diagnosed by Ohene et al. (20); detailed that 53.7% of grade III, 31.5% of grade II and 14.8% of grade I. Essiben et al. showed a propensity in the following course of incidence: grades II, I besides III to the Yaoundé Gynecological Obstetric and Pediatric Hospital (21)which is a referral centre for gynecological malignancies. METHODS: It was a retrospective descriptive study over a period of four years, from June 1(st. Koffi et al. presented that tumor grade II exemplified 58.4% of females, however grade I in addition II were in the comparable amount (20.8%) (22)which is a referral centre for gynecological malignancies. METHODS: It was a retrospective descriptive study over a period of

four years, from June 1(st. These results are conflicting to those reported by Meye et al. that, the grade II detained the past location after grade I and III (23)which is a referral centre for gynecological malignancies. METHODS: It was a retrospective descriptive study over a period of four years, from June 1(st.

According to association between ages groups and behavior of lesion either benign or malignant, there is significant association between age groups and cancer of breast, 37% of females in age group 41- 50 years old with malignant breast cancer, while 20% of malignancy in age group (31 – 40) and (51 – 60) years old. 13% of malignancy in age group 61 – 70 years old and only 6% in female over 71 years old age. Augustin et al. stated the same results that most patients in ages of 35 to 44 and 45 to 54 with 28.2% and 29.3% correspondingly. We can roughly that the major people found is dependable with some preceding studies approved globally (24, 25). Another study done by Hai-long Chen et al. showed that age groups from 50-59 years old was most group with breast cancer (26.4%), less than 30 years old and less than 40 years old represented low mount of patients (6.4%) (26). In another study stated that 6.4% of patients with breast cancer younger than 40 years old while 93.6% of them more than 40 years old, so the incidence of females to developed breast ca. was more in age group more than 40 years old so all women must do routinely breast cancer screening (27).

Conclusion

After pathological assessment of biopsies, malignant breast cancer (IDC/ grade II, IDC/ grade III, ILC and fibrocystic changes) most common changes, significant association between age group and breast cancer development more age group 41- 50 years old and then (31- 40), (51– 60) years old respectively.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

References

1. Shaukat U, Ismail M, Mehmood N. Epidemiology, major risk factors and genetic predisposition for breast cancer in the Pakistani population. Asian Pac J Cancer Prev. 2013;14(10):5625–5629.
2. Ly M, Antoine M, André F, Callard P, Bernaudin JF, Diallo DA. Breast cancer in Sub-Saharan African women. Bull Cancer. 2011;98(7):797–806.
3. Elgaili EM, Abuidriss D, Rahman M, Michalek AM, Mohammed SI. Breast cancer burden in central Sudan. Int J Womens Health. 2010;9(2):77–82.
4. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. LOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 11 [Internet] Lyon, France. In: *International Agency for Research on Cancer.* globocan.iarc.fr; 2013.
5. Naderimagham S, Alipour S, Djalalinia S, Kasaeian A, Noori A, Rahimzadeh S, et al. National and sub-national burden of breast cancer in Iran; 1990–2013. Arch Iran Med. 2014;17(12):794–799.
6. Bray F, Ren JS, Masuyer E, Ferlay J. Global estimates of cancer prevalence for 27 sites in the adult population in 2008. Int J Cancer. 2013;132(5):1133–1145.
7. Majeed W, Aslam B, Javed I, Khaliq T, Muhammad F, Ali A, et al. Breast cancer: major risk factors and recent developments in treatment. Asian Pac J Cancer Prev. 2014;15(8):3353–3358.
8. Cole P, Mark Elwood J, Kaplan SD. Incidence rates and risk factors of benign breast neoplasms. Am J Epidemol. 1978; 108:112–20.
9. Hutchinson WB, Thomas DB, Hamlin WB, et al. Risk of breast cancer in women with benign breast lesion. J Natl Cancer Inst. 1980; 65:13–20.
10. Mansel RE. Benign breast disease. Practitioner. 1982; 232:830–37.
11. Sainsbury RC. Bailey and Love's Short Practice of Surgery. 25th. London: Edward Arnold Ltd.; 2008. Breast In: Norman WS, Bulstrode CJK, P.Ronan O'Connel editors; pp. 827–35.
12. Sangma MB, Panda K, Dasiah S. A clinico-pathological study on benign breast diseases. *J Clin Diagn Res*. 2013;7(3):503–506.
13. Allred DC. Ductal carcinoma in situ: terminology, classification, and natural history. J Natl Cancer Inst Monogr. 2010; 2010: 134-138.
14. Erbas B, Provenzano E, Armes J, Gertig D. The natural history of ductal carcinoma in situ of the breast: a review. Breast Cancer Res Treat. 2006; 97: 135-144.
15. Sanders ME, Schuyler PA, Dupont WD, Page DL.

The natural history of low-grade ductal carcinoma in situ of the breast in women treated by biopsy only revealed over 30 years of long-term follow-up. Cancer. 2005; 103: 2481-2484.

16. AMERICAN JOINT COMMITTEE ON CANCER. AJCC Cáncer Staging Manual - 8th Edition. 2017. 1–1001 p.
17. Balekouzou A, Yin P, Pamatika CM, et al. Epidemiology of breast cancer: retrospective study in the Central African Republic. *BMC Public Health*. 2016;16(1):1230. Published 2016 Dec 7.
18. Yu, Z. G. *et al.* The prevalence and correlates of breast cancer among women in Eastern China. *PLoS ONE* **7,** (2012).
19. Spivey GH, Perry BW, Clark VA, Coulson AH, Coulson WF. Predicting the risk of cancer at the time of breast biopsy. Variation in the benign to malignant ratio. Am Surg. 1982 Jul;48(7):326–32.
20. Ohene-Yeboah M, Adjei E. Breast cancer in Kumasi, Ghana. Ghana Med J. 2012 Mar;46(1):8–13.
21. Essiben F, Foumane P, Mboudou ET, Dohbit JS, Mve Koh V, Ndom P. [Diagnosis and treatment of breast cancer in Cameroon: a series of 65 cases]. Mali Med. 2013;28(1):1–5.
22. Koffi B, Sepou A, Doui DA, Goumba C, Djabanga S. Epidemiological and histological characteristics of breast cancers in Bangui [French article] Med Afr Noire. 2004;1(2):112–4.
23. Meye JF, Ngomo KM, Diallo I. Breast cancer hospital in Libreville [Article in French] Med Afr Noire. 2004;51(8–9):479–72.
24. Mobima T, Ouiamon R, Koandongui F, Glamende, Koffi B. Histo-sonographic correlation of breast lesions in Bangui (CAR) [French article] J Afr Imag Med. 2015;4(7):431–438.
25. Adebamowo CA, Ayayi OO. Breast Cancer in Nigeria. West Afr J Med. 2000;19:179–181.
26. Chen, H. L., Zhou, M. Q., Tian, W., Meng, K. X. & He, H. F. Effect of age on breast cancer patient prognoses: A population-based study using the SEER 18 database. PLoS ONE 11, (2016).
27. Gnerlich JL, Deshpande AD, Jeffe DB, Sweet A, White N, Margenthaler JA. Elevated breast cancer mortality in women younger than age 40 years compared with older women is attributed to poorer survival in early-stage disease. Journal of the American College of Surgeons. 2009;208(3):341–7.

A Histological and Histochemical Study on Olfactory Bulbs to Detection Amyloid Protein Depositions by Congo-Red and Routine Staining Techniques

Rajaa Ali Moheiseen Al- Taee[1], Muna Hussain Al-Aameli[2], Yarub Modhar Al-Qazwini3

[1]Medical dep. / Hammurabi Medical Collage / University of Babylon/Iraq, [2]Collage of Veterinary Medicine / Kerbala University/Iraq, [3] Collage of Education for Pure Science / Kerbala University/Iraq.

Abstract

In this study, histological and histochemical techniques were used to examine olfactory bulbs in the albino rat male. Thirty male albino rats were split into three age groups (10 animals each) in the current research: **Group I**: consider as a control group, including adult animals aged 3 months .**Group II:** include animals aged 6 months. **Group III**: include animals aged 12 months. The histological architecture of the layers of olfactory bulbs and their main cells was identified by using H & E staining techniques, meanwhile the composition of each layer in albino rat was evident. In the glomerular and mitral cell layer of group III, olfactory bulbs showed reduced neural density. Modified staining with Congo - red was conducted for histochemical studies. Compacted amyloid cores were found in group III animals' olfactory bulbs, while dispersed amyloid cores were found in group II&III olfactory bulbs' cortex. The present study adds to our knowledge of the impact of amyloid protein on olfactory bulbs and their prospective neurodegeneration involvement.

Key words: *Histology, Histochemistry, Congo red, Amyloid ,Granular cells, Mitral cells and olfactory bulbs.*

Introduction

In the olfactory signal conductive path, the olfactory bulbs are the station of relay. The OBs are the first chief relay in the processing of odor data process: it provides afferent input from olfactory sensory neurons (OSN) in the olfactory epithelium of the nasal cavity and responsible for the identification of odors in it: which is the only relay between the peripheral and central nervous system, it also processed the olfactory data [(5)]. The olfactory bulb's main histological architecture involve: olfactory nerve layer, glomerular olfactory layer, external plexiform layer, mitral cell layer, inner plexiform layer and granular cell layer [(8)]. The superficial olfactory nerve layer contains afferent axons from OSNs. OSN axons synapse with OB projections of neurons dendrites, mitral / cells, and sub aggregations of regional periglomerular cells (Pgm) [(13)]. These layers are arranged in the olfactory bulbs very obviously and regularly. The characteristics of olfactory bulbs structure facilitate the processing of data and also provide a scaffold for olfactory data spatial encoding [(5)].

The olfactory bulb consists of four types of cells: mitral cells, granular cells, tufted cells and short axon cells according to classical studies [(10; 5)]. One of these neurons, granular cells, have long been known to be morphologically uncommon because they don't have a standard axons, and recent studies in electron microscope have demonstrated that it participate in special reciprocal synaptic connections with mitral cells [(8)].Mitral cells are the biggest cells in the olfactory bulbs and as stated by electron and light microscope researches, moreover they are the main olfactory bulb efferent neuron [(6)]. The dendritic structures of mitral cells can be subdivided into primary and secondary dendrites, and both primary and secondary smooth dendrites pass through the surface of the outer plexiform layer and only the primary dendrites extend down to the olfactory glomerular layer [(18)]. Within the glomerular layer the dendrites of mitral cells are in synaptic communication with olfactory nerves and periglomerular cells, but the only synapses on mitral cells elsewhere are the "reciprocal synapses'' with the granule cells [(5)]. Mitral cells, as the main olfactory bulbs efferent neurons, play a significant role in olfactory signal conduction and modification [(9; 13)].

Amyloids β (AB) are little pieces of a bigger proteins named "amyloid precursors protein" APP. While the ordinary function of APP has not yet been determined by researchers, they understand a lot about how it appears to operate [16]. In its full shape APP ranges from the inside of the brain cells to the outside through the fatty membrane around the cells. As APP is activated to perform its normal activities, other protein can cut it into different and smaller parts inside and outside cells. APP can be cut in several aspects: under some conditions, β-amyloid is one of the parts generated [3].

According to the hypothesis of amyloid, phases of beta-amyloid aggregations interrupt cell-to-cell communication and activate immune cells, these cells of immune system cause inflammation [12].

The process of neurodegeneration in diseases of cognition (Alzheimer's and Parkinson's diseases) may involve toxicity of β-amyloid (Aβ) that could be demonstrated in vitro and seems to be involving oxidative stress, this underlie the progression of neurodegeneration that consider as characteristic feature of AD [2].

It has been shown that the amyloid induces neuronal death, reduced plasticity of synapses, aberrant axons growth, tau hyper phosphorylation and chronic inflammation [7] . Aβ accumulation in the pathogenesis of AD is an early and essential case .First formation of temporal cortical regions, including the hippocampus, a memory-creating zone. Aβ aggregates have been indicated to form neurotoxic plaques that contribute to neurodegeneration accompanied by dementia [15].

Similarly, [11)] showed that capillaries, venioles and arterioles in the cerebral cortex also often have amyloid deposits. Congo red staining was considered as an approved histochemical marker for amyloid β-pleated-sheet [14]. Congo red: amyloid detection in tissue parts is significantly improved and verified by favorable Congo red staining. Thioflavin S and Congo red are represented the main histological stains that used for any type of amyloid [17].

Red stain is red-pink on its own .Under both light and polarized light microscopy, examination of tissue segments suspected of participation by amyloidosis must be carried out. Amyloidosis has a distinctive green apple birefringence when polarized[11].

In this study we analyzed the histological structure and histochemical (Congo red for amyloid protein) characteristics of OBs in order to evaluate the presence of amyloid depositions in the olfactory bulb layers. The aim of this research is to clarify the amyloid-histochemical and histological characteristics of olfactory bulbs in the rats of albino male in relation with aging.

Material and Method

Experimental Animals:

Male albino rats aged between (3, 6, 12) months were obtained from the Animal House, Collage of Science, University of Babylon. The rats were housed in wire mess cages under standard condition with 12 hrs. Light and 12 hrs. dark cycle throughout the entire experimental period. Food and tap water supplied with libitum.

In the current research, thirty male albino rats will be split into three age groups (10 animals each), they are: Group I: considered as a control group, including adult animals aged 3 months. Group II: include animals aged 6 months. Group III: include animals aged 12 months.

Histological study:

The specimens of olfactory bulbs were taken from the brain of the albino rats, the samples were immersed in the solution of Bouins for two days. Tissue was dehydrated in graded ethanol and embedded in paraffin. 7μm horizontal sections of paraffin blocks were cut on a revolving microtome and installed on glass slides then, the following staining processes were completed [1].

Histochemical Study:

Selected sections have also been processed for histochemical amyloid protein demonstration. Sections have been deparaffinized through xylene and alcohol into tap water. Thereafter, slides are immersed in alkaline sodium chloride. Twenty minutes later, they were immersed in alkaline solution of Congo red and then marked with alcoholic potassium. Thereafter, slides are countered with alum hematoxylin and dehydrated by xylene and ethanol [6].

Results

Histological Study:

In light microscopy, the main olfactory bulbs of the adult rat consisted of six concentrated layers: 1-The olfactory nerve layer (ONL), (fig.1A, and 1B). 2- The glomerular layer (GML). There were observation of

periglomerular cell (Pgm) around the glomeruli (fig.1D, 1E). 3- The layer of the external plexiform layer (EPL), consists of fine nerve fibers and few granule cells, (fig.1A, 1B). 4- A layer of mitral cells (MCL) contained stomata of mitral cells in single row (fig.1A, 1B). They had an ovoid nuclei with single nucleolus deeply stained. Their cytoplasm include darkly stained basophilic granules (fig. 1C, 1E). 5-The inner plexiform layer (IPL), a thin layer of fine nerve fibers and some cells of granules (fig. 1A, 1B). 6- The layer of granule cells (GCL), contained a large amount of granule cells (fig. 1A, 1B).

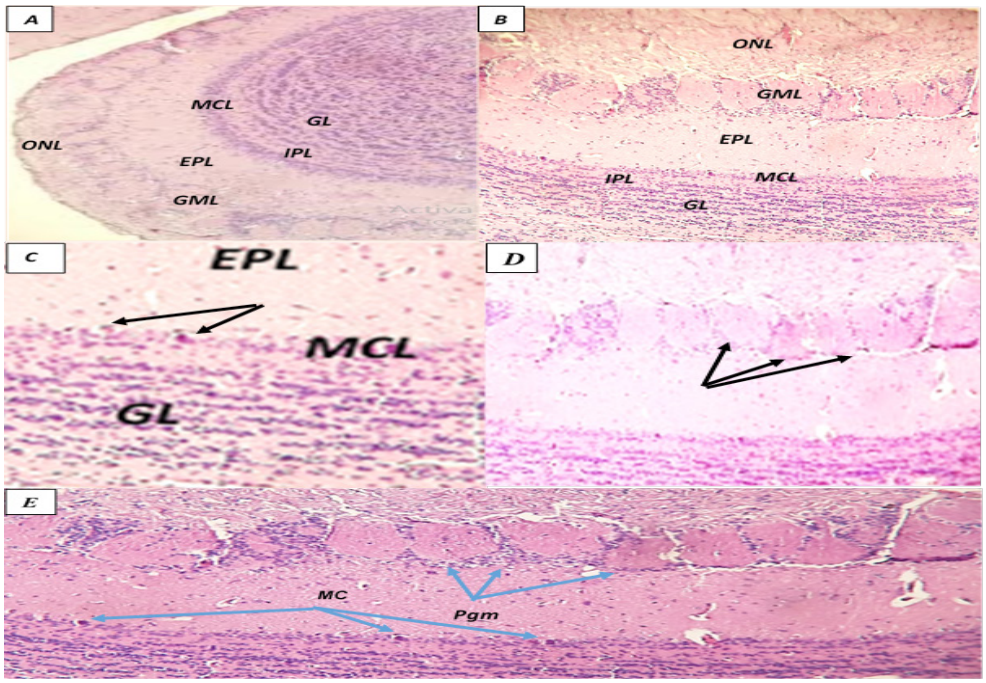


Fig.1: photomicrograph of histological section demonstrated to the concentric laminar organization of olfactory bulbs, H&E staining.4x, 10x, 40x.

The olfactory nerve fibers have been loosely segregated in group III rats. With a marked decrease in size, glomeruli were distorted in form (fig. 1). The EPL included a number of misplaced mitral cells' soma (fig.1B, 1E). Most mitral cells in MCL were decreased in size and generally present in a rounded, darkly stained nuclei with undefined nucleolus (fig.1C).

Histochemical Study:

Two forms of plaque of amyloid protein deposition were seen in the cortex of olfactory bulbs: diffuse plaques were shown in all layers of OBs of group III (fig. 2B). Compact amyloid plaques were found in the ONL, GML, MCL, and EPL layers of OBs of group III (fig. 2A, 2B). Amyloid angiophathy were detected in the different layers of OBs of group II and III, (fig.3).

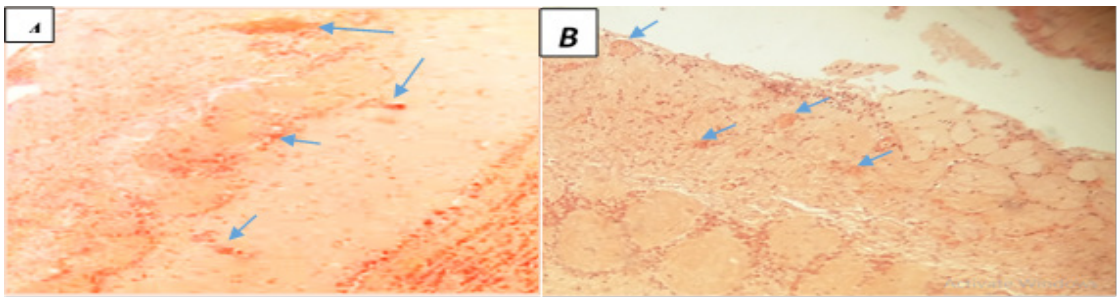


Fig.2: Photomicrograph of histological section showing plaque & diffuse staining for amyloid (AB, blue row) with Congo red –stain in olfactory tissue (2A, 2B), 10 x.Fig.3 (A,B): Photomicrograph of histological section illustrated the Congo red stain highlighted the vascular amyloid depositions, note the staining of vessels walls (black & blue arrow), 40 x.

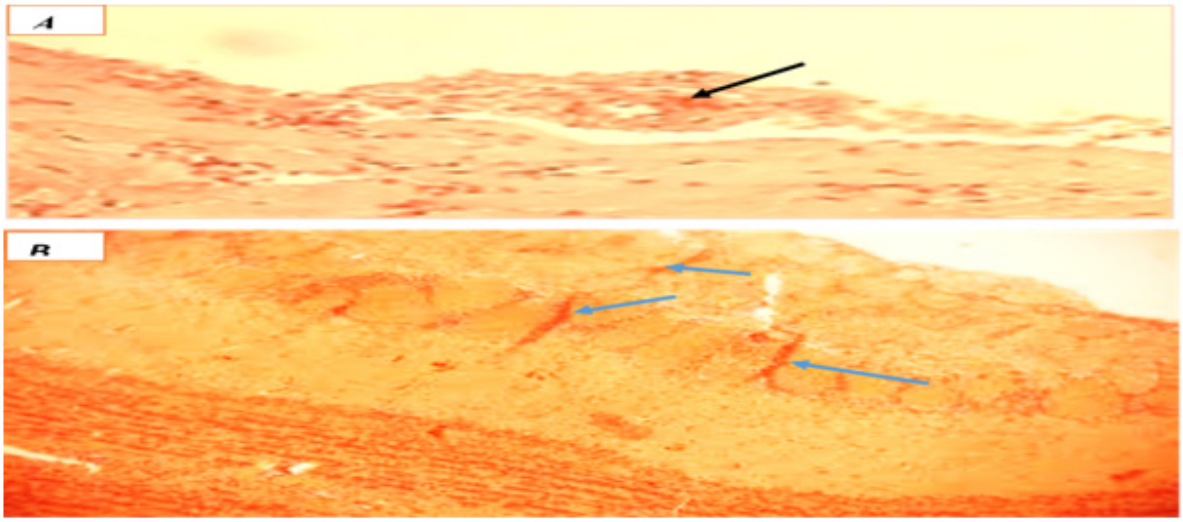


Fig.3 (A,B): Photomicrograph of histological section illustrated the Congo red stain highlighted the vascular amyloid depositions, note the staining of vessels walls (black & blue arrow), 40 x.

Discussion

In the pathway of the olfactory system, the olfactory bulbs are the essential components of olfactory system and relay station. We studied the histological architecture of the olfactory bulbs, according to the outcomes of our research, surveys were done to the structure of each layer of bulbs. The histological characteristics features of OBs constituent with the prior studies by Golgi [10]. Many periglomerular (Pgm) cells were found in the layer of glomeruli of olfactory bulbs. Two groups of neuronal cells are dispersed in the layer of mitral cells of olfactory bulbs, the cells with large cell bodies and cells with small cell bodies. The cells that characterized by large cell bodies seem to be correspond to the mitral cells as output neuronal cells, and the cells with small cell bodies may related to the tufted cells, that are well known as other kind of neurons of OBs of mammals, or the interneurons in the layer of mitral cells, but the correspondence could not be determined [5].

According to the finding of the present work, the histological characters among the bulbs of olfactory of albino rat are comparable in all groups (I, II, III), but the density of cells in each group was different. The complexity of the olfactory bulbs layers organization proportional to the olfactory bulbs information-processing capacity and represents the degree of olfactory bulb development. The results of our outcomes showed that the morphology and amount of mitral cells in the group I &III were differed [18].

The results of this research are complementary and consistent with prior human OBs tissue reports that prevalent layers (NFL, GML, EPL, MCL, IPL, and GL) constitute the construction of all layers in the olfactory bulbs of albino rats. There was no distinction in olfactory bulb composition between group (I, II, III), except for cell density variations. In group I, in each layer, the density of cells was higher than in group II and III. No mechanism exited to explain this decline. The amount of granulated cells and mitral cells decreased but increased in size [11; 9]. The mitral cells considered as the largest neuronal cells in the olfactory bulbs, have primary and secondary dendrites, these processes oriented vertically or parallel to their soma, glial cells formed these dendrites of mitral cells. Axons of mitral cells converge in bundles of fibers and pass through the layer of granular cells [11].

[10] Demonstrated a "substantial layer-specific loose" of synapses ultra-structurally: synaptic density is decreased in the layer of glomerular cells but not the internal plexiform layer, leading to unbalance in circuitry of OBs. Our findings showed a decrease in GML and MCL density, consistent with [10] findings, these results indicate that decreased afferent synaptic input and local modulatory circuit synapses in OB glomeruli may contribute to particular age related changes in olfactory function.

In the current research, we showed diffusing and plaques of compact amyloid nuclei in the cortex of OBs by using modified Congo red staining. We also showed the enhanced amount of plaques and reduced neuronal populations in group III OBs compared to the olfactory cortex of group I& II, that showed typical dark orange colored patches under light microscope. It is well known that there are plenty of extracellular plaques of amyloid β peptide (*A*β) in the pathological marks of AD in the brain [(16)]. Amyloid has been discovered to be more localized in the neuronal processes in the current research, this finding was in agreement with the outcomes of other researchers who noted that in elderly people with and without Alzheimer's disease ,abnormality amyloid accumulate as neuropil threads [(11)]. The method of neurodegeneration in AD may involve toxicity of β-amyloid (*A*β). *A*β's neurotoxicity can be shown in vitro and seems to involve oxidative stress [(2)].

Our finding demonstrated that the blood vessels within the cortex of olfactory bulbs also lades depositions of amyloid that constituents with the results of [(14)]. Congophilic amyloid in blood vessels is called cerebral amyloid antipathy (CAA) [(17)].

Many researchers have shown that the deposition of *A*β peptide in the cerebral cortex leads in neural and morphological degeneration, cognitive loss, and modulation of enzyme markers such as acetylcholine esterase and choline acyltransferase; all of which are well-known symptoms of AD [(19; 4)].

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

References

1. Bancroft J. D. and Stevens A. Theory and Practics of Histological Techniques. 7th edition Chirchill Livingstone 2012; 127-129.
2. Behl, C., D., J.B., Lesley, R. and Schubert, D. Hydrogen peroxide mediates amyloid beta protein toxicity. Cell. 1994; 77: 817-827.
3. Greenwald and Riek .Biology of Amyloid: Structure, Function, and Regulation, Zurich, Switzerland, Structure 18, 2010; October 13@2010 Elsevier, DOI 10.1016/j.str.2010.08.009.
4. Hashimoto, M., Hossain, S., Shimada, T., Sugioka, K., Yamasaki, H., Fujii, Y., Ishibashi, Y., Oka, J. and Shido, O. Docosahexaenoic acid provides protection from impairment of learning ability in Alzheimer's disease model rats. *J. Neurochem.* 2002; *81: 1084-1091.*
5. Huart C.; Rombaux PH. and Hummel Th. Plasticity of the Human Olfactory System: The Olfactory Bulb. Molecules, 2013; 18, 11586-11600; doi: 10.3390/molecules180911586. ISSN 1420-3049. ww.mdpi.com/journal/molecules.
6. Kohl Z. , Schlachetzki J. C. M., Feldewerth J, Hornauer P, Münch M., Adame A., Riemenschneider M. J., Winkler J., and Masliah E. Distinct Pattern of Microgliosis in the Olfactory Bulb of Neurodegenerative Proteinopathies. Hindawi Neural Plasticity, 2017; Volume 2017, Article ID 3851262, 15 pages,https://doi.org/10.1155/2017/3851262
7. Lo´pez-Toledano, M.A. and Shelanski, M.L. Neurogenic Effect of β-Amyloid Peptide in the Development of Neural Stem Cells. *J. Neuroscience,* 2004; *24 (23): 5439-5444*.
8. Mirich JM1, Williams NC, Berlau DJ, Brunjes PC. Comparative study of aging in the mouse olfactory bulb. J Comp Neurol. 2002; 454(4):361-72.
9. Mombaerts P .Axonal wiring in the mouse olfactory system. Annu Rev Cell Dev Biol , 2006; 22:713–737.
10. Nagayama S., Homma R. and Imamura F. Neuronal organization of olfactory bulb circuits. September Frontiers in Neural Circuits, 2014; 8(98):98.
11. Nobakht M. ; Seyed Mohammad Hoseini ; Pejman Mortazavi ; Iraj Sohrabi1; Banafshe Esmailzade ; Nahid Rahbar Rooshandel1and Shila Omidzahir. Neuropathological Changes in Brain Cortex and Hippocampus in a Rat Model of Alzheimer's Disease , Iranian Biomedical Journal, 2011; 15 (1 & 2): 51-58 (January & April 2011).
12. Rajamohamedsait H. B. and Aut E. M. S. Histological Staining of Amyloid and Pre-Amyloid Peptides and Proteins in Mouse Tissue. Methods Mol Biol. 2012; 849: 10.1007/978-1-61779-551-0_28. doi: 10.1007/978-1-61779-551-0_28.
13. Richarda M. B. , Taylora S. R., and Greera C. A. Age-induced disruption of selective olfactory bulb synaptic circuits, Proc Natl Acad Sci U S A. 2010; 107(35): 15613–15618. Published online

2010 Aug 2. doi: 10.1073/pnas.1007931107 PMCID: PMC2932573 PMID: 20679234 From the Cover Neuroscience

14. Verbeek MM, Vinters H, and de Waal RM. Cerebrovascular Amyloidosis in Alzheimer's Disease and Related Disorders. *Amsterdam: Kluwer,* 2000; *In press.*
15. Wallenstein, G.V.H. and Hasselmo, M.E. The hippocampus as an associator of discontinuous events. Trends Neurosci. 1998; 21: 317-323.
16. Wan L., G. Nie, J. Zhang, and B. Zhao Overexpression of human wild-type amyloid-β protein precursor decreases the iron content and increases the oxidative stress of neuroblastoma SH-SY5Y cells," Journal of Alzheimer's Disease, 2012; 30(3) 523–530.
17. Wilcock D. M.; Gordon M. N. & Morgan D. Quantification of cerebral amyloid angiopathy and parenchymal amyloid plaques with Congo red histochemical stain. 2006;Available from: https://www.researchgate.net/publication/6416405 _
18. Yokosuka M., Hagiwara A., Saito T. R., Tsukahara N., Aoyama M., Wakabayashi Y., Sugita S. and Ichikawa M. Histological Properties of the Nasal Cavity and Olfactory Bulb of the Japanese Jungle Crow Corvus macrorhynchos. Chem. Senses 2009; 34: 581–593. doi:10.1093/chemse/bjp040 .Advance Access publication July 20, 2009.
19. Yun Fan L.; Kai-Yuan Tzen ; Ya-Fang Chen6 ; Ta-Fu Chen1 ; Ya-Mei Lai1 ;Ruoh-Fang Yen ; Ya-Yao Huang ; Chyng-Yann Shiue ; Shieh-Yueh Yang and Ming-Jang Chiu .The Relation Between Brain Amyloid Deposition, Cortical Atrophy, and Plasma Biomarkers in Amnesic Mild Cognitive Impairment and Alzheimer's Disease, Frontiers in Aging Neuroscience, 2018; Volume 10 Article 175.

The Intraperitoneal-Ketoprofen-Histopathological Induced Alterations in the Wistar Rat Kidneys

Samer Riyadh Fadhi[1], Hakim Bahlok Jebur[2]

[1]Department of Pathology and Forensic medicine, College of Medicine, Wasit University, Iraq,
[2]Department of Pharmacology, College of Medicine, Wasit University, Iraq

Abstract

The current-focused study was carried out to generate a data profile about expected changes that could be induced in the kidneys of rats due to the use of intraperitoneal ketoprofen (KP). The study involved using 24 adult male Wistar rats sorted randomly into four groups (six animals per group). One group was treated as a control group, C, which was supplied with distilled water (DW) only. The remaining animals were presented as (50KP), (25KP), or (12.5KP) groups that received 50mg/kg.b.w., 25mg/kg.b.w., or 12.5mg/kg.b.w., respectively, of KP. The experiment was continued for 70 days, and the kidney tissue samples were collected from the scarified animals at the end of day 70 of that experiment. The kidney tissues of the 50KP animals revealed dilation of the tubules throughout the outer strip of the outer medulla with necrosis and sloughing of the epithelial cells of the proximal convoluted tubules (PCTs). However, the 25KP group suffered lesser grades of epithelial-cell sloughing of the PCTs with lower levels of dilation of tubules than those recorded in the 50KP group. On the other hand, the kidney tissues of the 12.5KP group showed only dilation in the PCTs. The present experimental data unveil the side effects generated by the use of the intraperitoneal KP in the examined rat kidneys which should be used as a launching set of information for better use or further study this drug and its side effects in human patients.

Keywords*: Ketoprofen, renal failure, side effects.*

Introduction

KP is an anti-inflammatory, anti-pyretic, analgesic derivative of propionic acid with non-steroidal anti-inflammatory (NSAID) therapeutic properties. KP reduces cyclooxygenase I and II enzyme activity, which contributes to a reduction in prostaglandin and thromboxan precursor production. As a consequence, the decline in prostaglandin production triggers the therapeutic potential of ibuprofen via the enhancement of prostaglandin synthase. The production of A2 thromboxane by thromboxane synthases, which prevent the accumulation of platelets, is also decreased with KP [(1)]. KP is the 11th most popular in Italy with 206 records for 2008, of which some 30 percent is extreme, based on a survey of random cases of adverse outcomes. A maximum of 13 percent of reported data were in the aspect of pediatric patients (under 18 years of age), even in the age group (under six years old) of off-label medication use. The 2012 evidence is not fully accessible; however, unofficial statistics show that KP was implicated in 560 adverse drug reactions, of which 31 percent were seriously affected [(2,3)].

KP records are that because of the unwise consumption of KP in the world countries despite multiple warnings about hepatotoxicity compared to other NSAIDs such as nimesulide. The KP's reported adverse events contain peripheral edema as a cardiovascular response, central responses such as drowsiness, headache, etc., dermatological responses including skin sensitiveness and photosensitization, blood based responses e.g. edema, platelet malfunction, etc., kidney, elevation of enzymes in the liver, gastrointestinal reactions for example vomiting, diarrhea, gastric ulcer and bleeding, etc. Additional studies have shown at least intermittent serum aminotransferase elevations ranging between one percent and two percent of the patients receiving KP. Even with product continuity, these can be

Corresponding author:
Samer Riyadh fadhil
Email: Sfadhil@uowasit.edu.iq

overcome. For less than one percent of cases, reported elevations of aminotransferase (more than threefold increased) exist. It is very unusual that liver damage with KP jaundice is clinically evident and only few cases have been recorded. The latency to onset of the symptoms is a quick process, sometimes appear within a few days. The trends of enzyme changes differ between hepatocellular and cholestatic. In some instances, immunoallergic symptoms (low level fever, rash) are observed, but are not usually prevalent and self-antibody development is uncommon [4,5]

The performance of adverse KP effects on the kidney have not been comprehensively studied from the prospective of tissue damages. According to that, the current-focused study was carried out to generate a data profile about expected changes that could be induced in the kidneys of rats due to the use of intraperitoneal KP.

Materials and Method

Animals and experimental design

The study involved using 24 adult male Wistar rats, weighed at 175-250gm with 10-14 weeks of age, sorted randomly into four groups (six animals per group) that lived in a housing under 22-25°C. One group was treated as a control group, C, which was supplied with distilled water (DW) only. The remaining animals were presented as (50KP), (25KP), or (12.5KP) groups that received 50mg/kg.b.w., 25mg/kg.b.w., or 12.5mg/kg.b.w., respectively, of KP. The experiment was continued for 70 days, and the kidney tissue samples were collected from the scarified animals at the end of day 70 of that experiment.

Kidney tissue preparation

Ten percent formalin was used for fixing the tissue specimens for two hours that was followed by a 30-minute-DW based removal step of the fixative. Then, a series of alcohol concentrations at (70% for 30mins, 90% for 60mins, and 100% as two cycles for 60mins per cycle) was used to dehydrate the tissues followed by a 50%:50% of alcohol to xylene immersing step for clearing the tissues for 60mins. After that, the tissues were immersed in a pure xylene for 90mins. Later, molten paraffin wax was used to impregnate the tissues followed by embedding and blocking out those tissues. Paraffin based sections at 4 to 5um were hematoxylin- and eosin-stained. The tissue sections were prepared according to [6–8].

Results

The kidney tissues of the 50KP animals revealed dilation of the tubules throughout the outer strip of the outer medulla with necrosis and sloughing of the epithelial cells of the PCTs, figure 1.

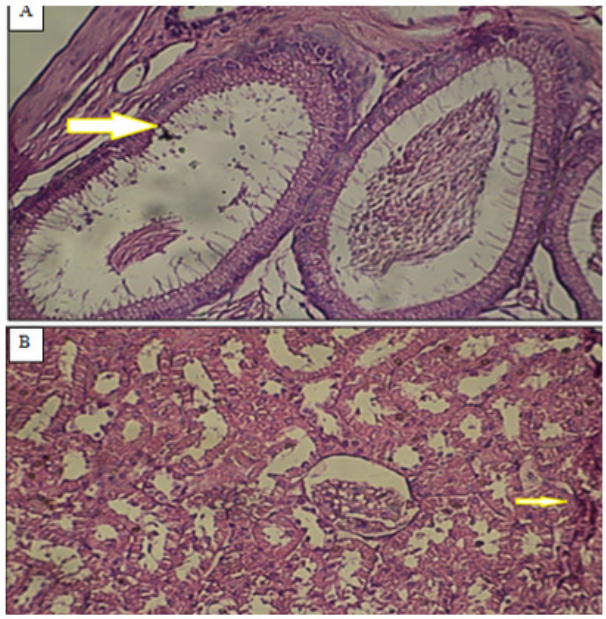


Figure 1: Rat kidney histopathological changes after intraperitoneal ketoprofen treatment at 50mg/kg.b.w. for 70 days. A. Dilation of the tubules throughout the outer strip of the outer medulla, (H&E) Stain.400X. B. Necrosis and sloughing of the epithelial cells of the proximal convoluted tubules. (H&E) Stain.200X.

However, the 25KP group suffered lesser grades of epithelial-cell sloughing of the PCTs with lower levels of dilation of tubules than those recorded in the 50KP group, figure 2.

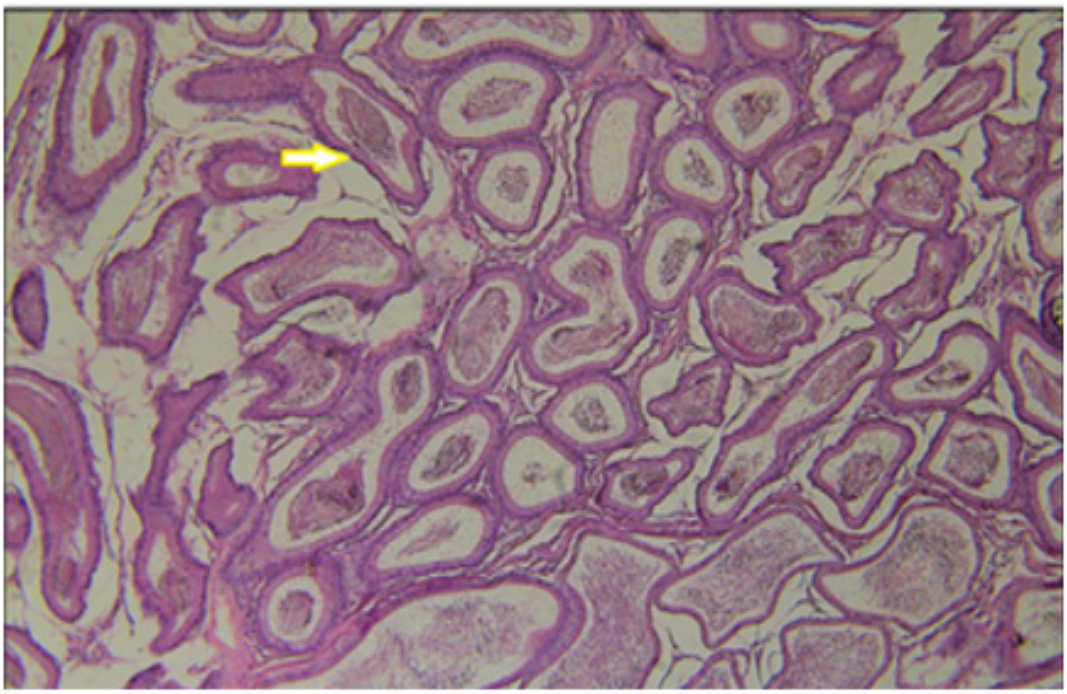


Figure 2: Rat kidney histopathological changes after intraperitoneal ketoprofen treatment at 25mg/kg.b.w. for 70 days. Changes are shown, here, as Lesser grades of epithelial-cell sloughing of the proximal convoluted tubules and lower levels of dilation of tubules than those recorded in the 50KP group. (H&E) Stain.200X.

On the other hand, the kidney tissues of the 12.5KP group showed only dilation in the PCTs, figure 3. The KP group kidney tissues were compared to each other and with the control group, figure 4.

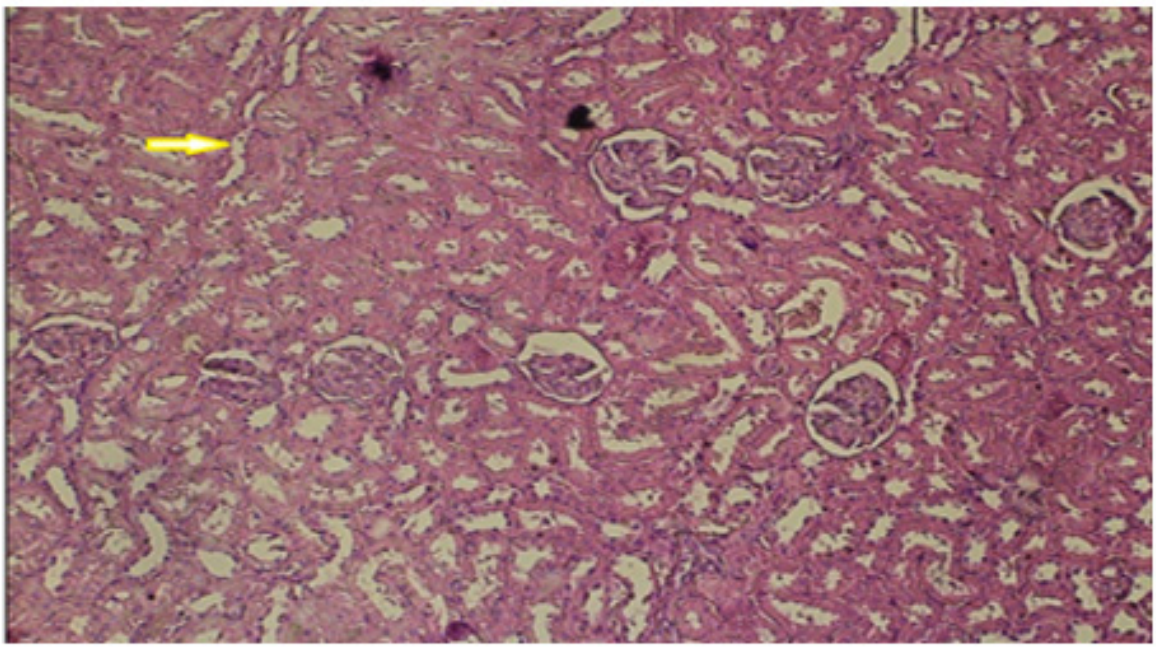


Figure 3: Rat kidney histopathological changes after intraperitoneal ketoprofen treatment at 12.5mg/kg.b.w. for 70 days. Only dilation in the proximal convoluted tubules is shown. (H&E) Stain.200X.

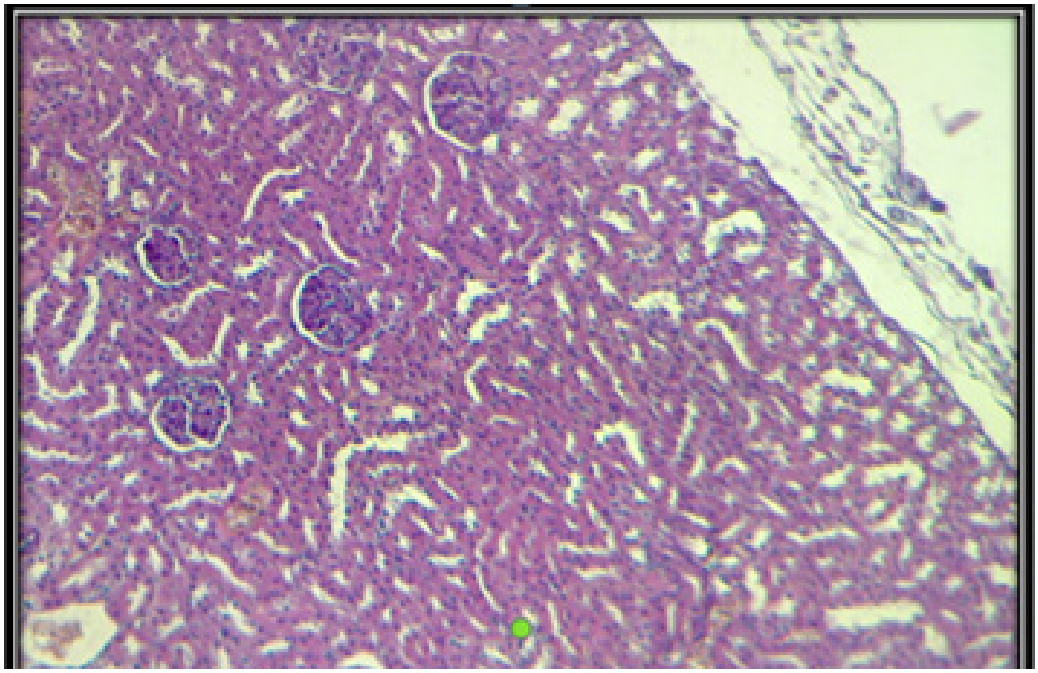


Figure 4: Normal cortical tubules and glomeruli of rat treated with distilled water within normal limits. (H&E) Stain.100X.

Discussion

Ketoprofen is a NSAID agent that is used for treating cases mediated by an inflammatory, fever, and/or pain process. The use of the drug as a therapeutic compound has been faced with a wide range of difficulties presented by the appearance of various adverse effects such as peripheral edema and platelet malfunction, drowsiness and headache, skin sensitiveness and photosensitization, and gastric ulcer and bleeding due to cardiovascular, central, dermatological, and gastrointestinal reactions, respectively, [9–13]. The adverse KP changes occurred in the kidney have not been fully sorted out. Therefore, the current work was conducted to characterized any histopathological alterations that could happen as responses to the use of KP in three concentrations.

The outcomes of the study unveiled that intraperitoneal ketoprofen treatment at 50mg/kg.b.w. for 70 days demonstrated dilation of the tubules throughout the outer strip of the outer medulla and necrosis and sloughing of the epithelial cells of the PCTs. Ingrasciotta *et al.*, [14] has found that using NSAID drugs such as oxicams, ketorolac, meloxicam, and piroxicam was positively correlated with the increased risk of developing chronic kidney disease (CKD). It has been suggested that utilizing ketorolac may induce CKD with a subclinical property due to acute renal damages [14]. This indicates an agreement with current findings that revealed the adverse effects encouraged by the use of the NSAID, KP, in the studied rats. The adverse effects of the KP use in humans can be inferred from a case report of a Turkish woman who received a topical treatment of KP as two times daily for five days who revealed increases in the levels of serum creatinine and urea which suggested an acute renal failure condition in this women [15]. The use of KP in pregnant women especially a short time before delivery has been found to increase the risk of renal dysfunction in the neonates[3]. The systemic-NSAID based kidney damages can be induced via acute interstitial nephritis due to a dose-independent allergic mechanism with cyclooxygenases 1- and 2-non-selective disruption causing an acute renal failure with reversed functions of the affected tissues [15].

However, those damages in the rat kidneys were correlatively decreased as the KP concentration was reduced. This was completely seen with groups 25KP and 12.5KP that showed lower grades of kidney tissue changes suggesting safer use of the KP with reduced concentrations. It has been recognized that using low doses of KP in children had led to the development of low rates of intense adverse effects with only nausea and vomiting [3].

The present experimental data unveil the side effects generated by the use of the intraperitoneal KP in the examined rat kidneys which should be used as a launching set of information for better use or further study this drug and its side effects in human patients.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding.

References

1. Kinney RG, Tjutrins J, Torres GM, Liu NJ, Kulkarni O, Arndtsen BA. A general approach to intermolecular carbonylation of arene C-H bonds to ketones through catalytic aroyl triflate formation. Nat Chem. 2018 Feb 1;10(2):193–9.
2. Gallelli L, Colosimo M, Pirritano D, Ferraro M, De Fazio S, Marigliano NM, et al. Retrospective evaluation of adverse drug reactions induced by nonsteroidal anti-inflammatory drugs. Clin Drug Investig [Internet]. 2007 [cited 2020 Feb 15];27(2):115–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17217316
3. Carbone C, Rende P, Comberiati P, Carnovale D, Mammì M, De Sarro G. The safety of ketoprofen in different ages. Vol. 4, Journal of Pharmacology and Pharmacotherapeutics. Wolters Kluwer -- Medknow Publications; 2013. p. S99.
4. Sardana V, Burzynski J, Zalzal P. Safety and efficacy of topical ketoprofen in transfersome gel in knee osteoarthritis: A systematic review. Vol. 15, Musculoskeletal Care. John Wiley and Sons Ltd; 2017. p. 114–21.
5. Miles S. Ketoprofen. In: xPharm: The Comprehensive Pharmacology Reference. Elsevier Inc.; 2007. p. 1–7.
6. Ibrahim KE, Al-Mutary MG, Bakhiet AO, Khan HA. Histopathology of the liver, kidney, and spleen of mice exposed to gold nanoparticles. Molecules. 2018;23(8).
7. Agarwal SK, Sethi S, Dinda AK. Basics of kidney biopsy: A nephrologist's perspective. Vol. 23, Indian Journal of Nephrology. Wolters Kluwer -- Medknow Publications; 2013. p. 243–52.
8. Ahmed A, Al Tamimi DM, Isab AA, Alkhawajah AMM, Shawarby MA. Histological Changes in Kidney and Liver of Rats Due to Gold (III) Compound [Au(en)Cl2]Cl. PLoS One. 2012 Dec 27;7(12).
9. Naidoo V, Venter L, Wolter K, Taggart M, Cuthbert R. The toxicokinetics of ketoprofen in Gyps coprotheres: Toxicity due to zero-order metabolism. Arch Toxicol [Internet]. 2010 Oct [cited 2020 Feb 15];84(10):761–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20140424
10. Tomic Z, Milijasevic B, Sabo A, Dusan L, Jakovljevic V, Mikov M, et al. Diclofenac and ketoprofen liver toxicity in rat. Eur J Drug Metab Pharmacokinet [Internet]. 2008 [cited 2020 Feb 15];33(4):253–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19230600
11. Praskova E, Voslarova E, Siroka Z, Plhalova L. Comparison of acute toxicity of ketoprofen to juvenile and embryonic stages of Danio rerio Auditory View project Psychoacoustical measurements, auditory models and their application. View project [Internet]. 2014 [cited 2020 Feb 15]. Available from: www.nel.edu
12. Salgado R, Pereira VJ, Carvalho G, Soeiro R, Gaffney V, Almeida C, et al. Photodegradation kinetics and transformation products of ketoprofen, diclofenac and atenolol in pure water and treated wastewater. J Hazard Mater. 2013 Jan 5;244–245:516–27.
13. Rangasamy B, Hemalatha D, Shobana C, Nataraj B, Ramesh M. Developmental toxicity and biological responses of zebrafish (Danio rerio) exposed to anti-inflammatory drug ketoprofen. Chemosphere. 2018 Dec 1;213:423–33.
14. Ingrasciotta Y, Sultana J, Giorgianni F, Fontana A, Santangelo A, Tari DU, et al. Association of individual non-steroidal anti-inflammatory drugs and chronic kidney disease: A population-based case control study. PLoS One. 2015 Apr 16;10(4).
15. Krummel T, Dimitrov Y, Moulin B, Hannedouche T. Acute renal failure induced by topical ketoprofen. Br Med J. 2000 Jan 8;320(7227):93

Call for Papers / Article Submission

Indian Journal of Forensic Medicine & Toxicology has commenced publication since 2007. IJFMT will be published two times in a year.

Purpose & Scope: Indian Journal of Forensic Medicine & Toxicology is a peer reviewed six monthly Journal. It deals with Forensic Medicine, Forensic Science, Toxicology, DNA fingerprinting, sexual medicine and environmental medicine. It has been assigned International standard serial No. p-0973-9122 and e-0973-9130 **website: www.ijfmt.com**. This journal is also indexed with Index Copernicus (Poland).

The journal encourages research from theoretical perspectives, research reports of evidence based practice as well as praxis research work that focuses on the interface between theory and practice and how each can support the other. In addition, the journal strongly encourages reports of research carried out within or involving countries in the Asia- Pacific region.

Invitation to submit papers:
A general invitation is extended to authors to submit journal papers for publication in IJFMT.

The following guidelines should be noted:

1. The article must be send by E-mail in word only as attachment. Hard copy need not be send.
2. The article should be accompanied by a declaration from all authors that it is an original work and has not been sent to any other journal for publication.
3. References should be in Vancouver style.
4. As a policy matter, journal encourages articles regarding new concepts and new information.

Please submit paper in following format as far as applicable

1. Title
2. Names of authors
3. Your Affiliation (designations with college address), email id
4. Corresponding author- name , designations, address,email id
5. Abstract with key words
6. Introduction or back ground
7. Material and Methods
8. Findings
9. Discussion / Conclusion
10. Conflict of Interest
11. Source of Support
12. Ethical Clearance
13. References in Vancouver style.
14. Word limit 2500-3000 words, MSWORD Format, single file
15. Please. quote references in text by superscripting

See website for all details

Our Contact info:

Our Contact Info:

Institute of Medico-Legal Publications

Logix Office Tower, Unit No. 1704, Logix City Centre Mall
Sector- 32, Noida - 201 301 (Uttar Pradesh)
Ph. +91 120 429 4015, +91 9971888542
E-mail: editor.ijfmt@gmail.com, Website: www.ijfmt.com

Indian Journal of Forensic Medicine & Toxicology

CALL FOR SUBSCRIPTIONS

About The Journal

Print-ISSN: 0973-9122 Electronic - ISSN: 0973-9130 Frequency: Quarterly

"Indian Journal of Forensic Medicine & Toxicology" is a peer reviewed six monthly Journal. It deals with Forensic Medicine, Forensic Science, Toxicology, DNA fingerprinting, sexual medicine and environmental medicine. It has been assigned International standard serial No. p-0973-9122 and e-0973-9130. The Journal has been assigned RNI No. DELENG/2007/21789.

The Journal is indexed with Index Copernicus (Poland) and is covered by EMBASE (Excerpta Medica Database). The journal is also abstracted in Chemical Abstracts (CAS) database.

Journal Title	Print Only
Indian Journal of Forensic Medicine & Toxicology	**INR 9000**

NOTE FOR SUBSCRIBERS

- Advance payment required by cheque/demand draft in the name of **"Institute of Medico-Legal Publications"** payable at Noida, Uttar Pradesh.
- Cancellation not allowed except for duplicate payment.
- Claim must be made within six months from issue date.
- A free copy can be forwarded on request.

Bank Details

Name of account :	**Institute of Medico-Legal Publications Pvt Ltd**
Bank:	**HDFC Bank**
Branch	Sector-50, Noida-201 301
Account number:	**09307630000146**
Type of Account:	Current Account
MICR Code:	110240113
RTGS/NEFT/IFSC Code:	HDFC0000728

Please quote reference number.

Send all payment to

Institute of Medico-Legal Publications
Logix Office Tower, Unit No. 1704, Logix City Centre Mall
Sector- 32, Noida - 201 301 (Uttar Pradesh), Ph. +91 120 429 4015, +91 9971888542
E-mail: editor.ijfmt@gmail.com, Website: www.ijfmt.com

Registered with Registrar of Newspapers for India
(Regd. No. DELENG/2007/21789)

Published, Printed and Owned : Dr. R.K. Sharma
Printed : Printpack Electrostat G-2, Eros Apartment, 56, Nehru Place, New Delhi-110019
Published at: Institute of Medico Legal Publications Pvt. Ltd., Logix Office Tower, Unit No. 1704, Logix City Centre Mall Sector- 32, Noida - 201 301 (Uttar Pradesh) Editor : Dr. R.K. Sharma, Mobile: + 91 9971888542, Ph. No: +91 120 429 4015