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Contents

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Synthesis, Characterization and Photo-Kinetic Study of Diphenol Schiff Base and its Metal Complexes with (Co²⁺, Ni²⁺, Cu²⁺) Ions

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Abstract

In this research 3,3'-[benzene-1,4-diylbis (nitromethylidene)diphenol (ligand) was prepared from reaction of 1,4-phenylenediamine with 3-Hydroxy Benzoic acid. This ligand was investigated using GC-Mass, (¹H, ¹³C) NMR, FT-IR and UV-Vis spectral studies. Complexes of ligand with the transition metal ions (Co²⁺, Ni²⁺, Cu²⁺) were prepared and investigated by using the techniques FTIR, UV-visible, molar conductivity and magnetic susceptibility.

Molar ratio of the prepared complexes was 1:1 for Co²⁺, Ni²⁺ complexes and 2:2 for (Cu²⁺) complex that conformed as dimer complex. Cobalt, nickel and copper complexes data were in agreement with an octahedral geometry. Photo-kinetic study was achieved for the bivalent copper complex. Kinetic study showed that photo reaction of the copper complex was from the first order by indicating of complexes concentration with the neglected of solvent amount. The values of photo-reaction constant were calculated for copper complex, which was 0.017 min⁻¹.

Keywords: Schiff base complexes, DiPhenol Schiff base, Photo-kinetic study, Metal complexes

Introduction

Schiff's bases are one of important kind of organic compound. These compounds are classified as primary amine which contained carbonyl group and it is common in organic chemistry and inorganic coordination chemistry (1).

Transition metal complexes with Schiff base were achieved many important studies brief important application in different fields like catalytic activity in hydrogenation of alkanes (2), photochromic properties (2-4) as well as the stability of these compound to complexing with some toxic metals (3-5).

The high affinity of Schiff base to chelate with transition metal ions encourage the researchers to prepare different kind of these compounds with transition metal ions and achieved many studies with different field of chemistry (2).

Another application of Schiff's base complexes were the biological activity against the different bacteria and fungi as the 2-hydroxy naphthalene-1-carbaldehyde-1,2-bis-(P-amine phenoxy) ethane with Ni²⁺, Mn²⁺, Co²⁺, Cu²⁺ and Zn²⁺ which have been recorded antifungal activities. Beside that the ability of these complexes as catalysts in different medium such as biological systems (4). Many photochemical studies were accomplished on metal complexes with different organic ligand. The most common one of these complexes were Schiff's base complexes with first transition metal series (5).

Many photo-degradation studies on inorganic complexes were achieved. These complexes showed photo sensitivity for UV-visible light (6). Photo-degradation is one of the most important processes used to degrade organic pollutants, which are released by many factories as liquid waste. Both the researcher Andrei V Budruv and his colleagues were able to achieve the process of

photo-degradation for (B rhodamine) with the presence of Iron (III) and hydrogen peroxide and ultraviolet light. Other variables, such as pH, compound concentration, dye, hydrogen hydroxide, and light intensity, were studied on reaction efficiency. In this interaction, a temporary course was proposed to understand the mechanism of photo-dynamics of B-rhodamine(7).

Dipti Vaya and etal achieved a study of photo-catalytic reaction on red phenol dye with the presence of Copper, Cobalt and Iron complexes with thiocyanate and hydrogen peroxides. In the photo degradation reaction of red phenol dye, the rate of photo-reaction was evaluated and studied by following by Uv-visible spectra. According to other variations like hydrogen, complexes concentration, dye concentration, hydrogen peroxide amount and light intensity were achieved and estimated there effects on photo-degradation reaction for the dye. Additionally, mechanism of reaction was suggested (8).

Photo-degradation of cobalt complex with EDTA was achieved by Kamal Rekab

And his researcher team, which proved that the photo catalytic reaction released cobalt from it's complex to get the cation form of cobalt with changing the oxidation state of from Co+2 to Co+3. This study used Uv-light (9).

Experimental Part

This research achieved at Physical and inorganic chemistry laboratories

Materials:

1- All used material and solvents in thisworkwere provided from the two companies BDH and Fluka and used without any modification.

2- The measurements were done at Mustansiriyah University laboratoriesand Technology University laboratories.

Infrared (FTIR) spectra were recorded by using FTIR .8300 Shimadzu spectrophotometer in the frequency range of 4000-400cm⁻¹.

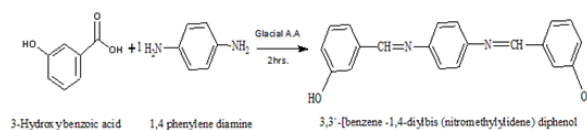
Electronic spectra using Varian UV-visible spectrophotometer and molar conductivity measurements using Philips conductivity meter.

The melting points were recorded in Coslab melting point apparatus.

The magnetic susceptibility of the solid complexes was obtained at room temperature using Magnetic Susceptibility Balance Johnson Matthey.

3- Preparation of the ligands:

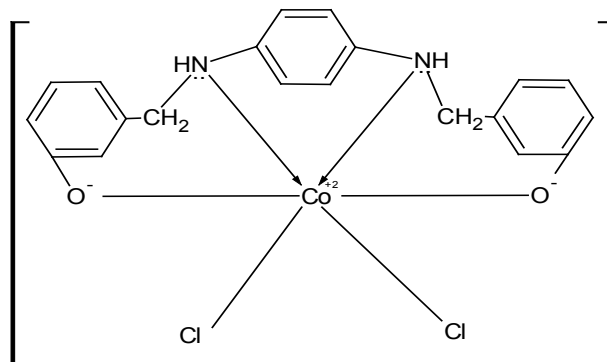
The ligands were prepared by the same method; A mixture of 3-Hydroxy benzoic acid(2mole)with 1mole of 1,4phenylene diamine by adding 2-3 drops of glacial acetic acid were refluxed for 2hours with continues stirring, after cooling at room temperature, the precipitate was filtered off, dried and recrystallized from ethanol(10) , (Scheme.1) referred to complexes preparation with the transition metal ions.



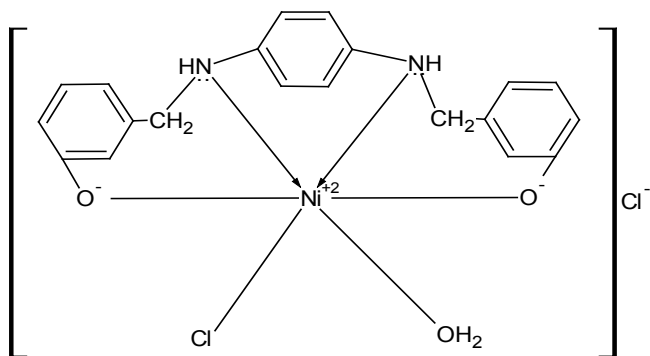
General equation of ligand preparation

4- Preparation of the complexes:

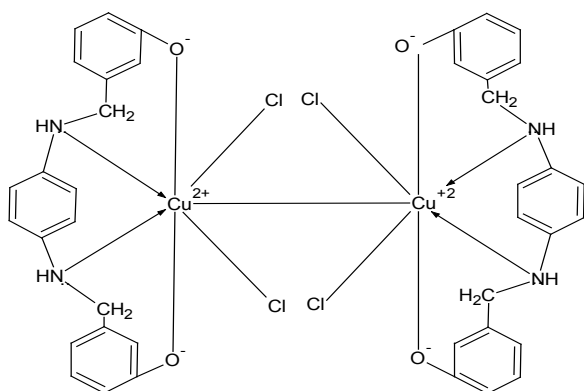
The complexes were prepared by dissolving ligand in 40ml absolute ethanol which then added drop wise with stirring to (1mmole) of MCl₂.XH₂O metal salts M=Co(II), Ni(II) , Cu(II) which were dissolved in 10ml of hot absolute ethanol. The mixture was heated to 70°C for 30min., then left overnight. The precipitated complex was filtered off, washed with 10ml cold ethanol and dried, scheme(2) referred to complexes structures.



Suggestion structure of cobalt (II) complex



Suggestion structure of nickel (II) complex



Suggestion structure of copper (II) complex

5- Photo-degradation study:

This study achieved according to the procedure at the literature (11). By which, three concentration of (1×10^{-4} , 4.0×10^{-4} and 0.5×10^{-4})M were prepared and exposure to UV-light for different time (15, 45, 75.....) min. Absorption of these radiated samples were recorded under constant conditions of temperature (25°C) and pressure.

Results and Discussion

¹H NMR characterization of prepared ligand (12-14).

¹H NMR data were documented by utilizing DMSO as solvent and the chemical shifts were transcribed in parts per million (ppm). The spectrum of the prepared ligand (Schiff base) gave a band at 10.13 (s, 1H, -OH(g)), 8.11 (s, 1H, CH=N(h)), 7.43-7.40 (d, 1H, b), 7.37-7.32 (t, 1H, c), 7.26 (s, 1H, f), 6.90-6.87 (d, 1H, d), 6.84-6.83 (d, 2H, i), 3.35±2.5 ppm → DMSO + water as shown at fig. 1.

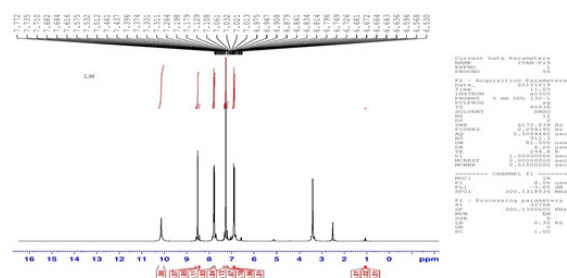


Fig.(1) : Shown ¹H NMR spectrum of the prepared ligand

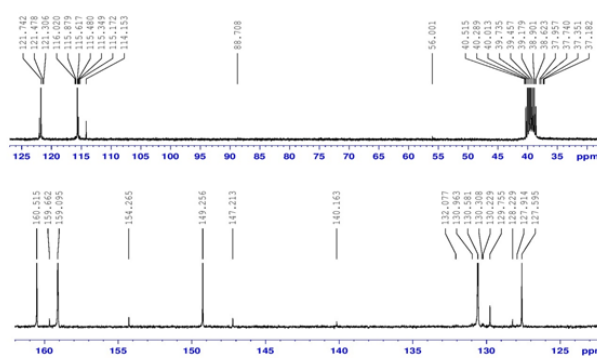


Fig.(2): ¹³C NMR Spectrum of the prepared ligand

Mass characterization of Ligand(15): Mass spectra of prepared ligand were calculated and its data were noted. The mass spectrum referred to main value of 316 which returned to the experimental formula of C₂₀H₂₀N₂O₂. Mass spectrum of the prepared ligand was illustrated at fig. 3.

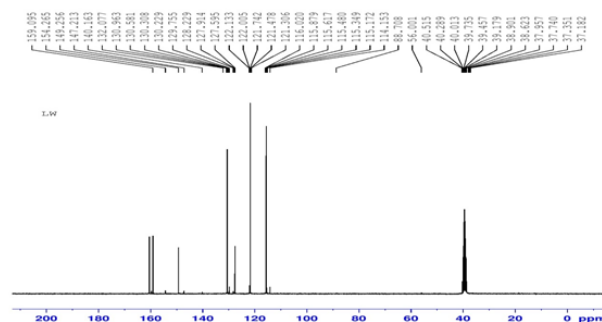


Fig.(3): Mass spectrum of prepared ligand.

Characterization of ligand:

The ligands and its metal complexes were insoluble in water but soluble in organic solvents such as DMSO, DMF and Ethanol, low conductivity values (8.6-69.7 cm²ohm⁻¹ mol⁻¹) indicated that the complexes are non-electrolytes and electrolytes [16]. The metal percentage in complexes analytical and physical data of ligands and complexes are given in Table (1).

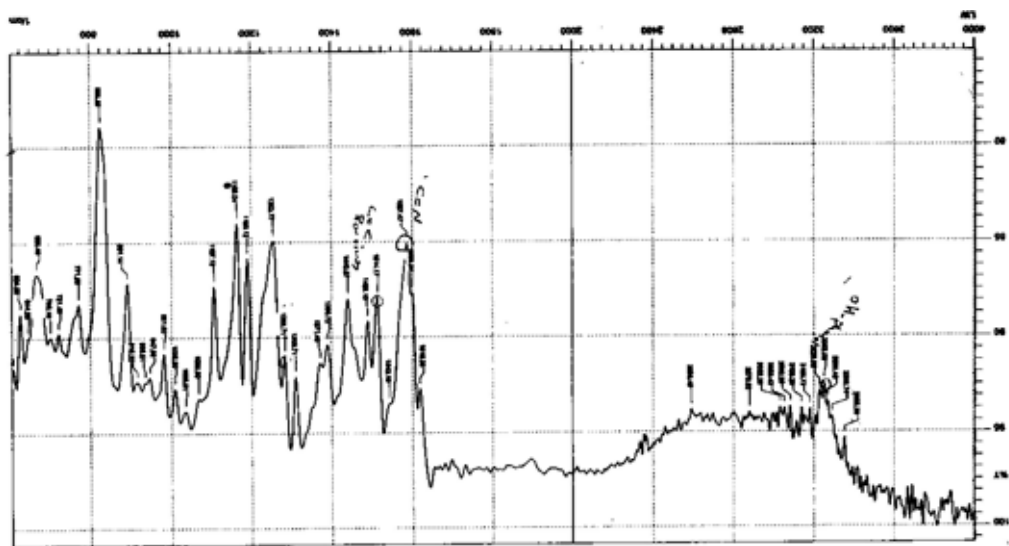
Table (1): Analytical and some physical data of ligand and its complexes.

M.L	yield%	M.p°C	Color	Molecular formal	symbol
----	65	215-217	Leaden	C ₁₆ H ₁₆ N ₂ 316	L
1:1	69	300*>	Red- brown	[CoL ₂ Cl ₂] 762	Co
1:1	81	300*>	Light green	[NiL(H ₂ O) ₂]Cl ₂ 762	Ni
2:2	78	290-292	Green	[Cu ₂ (L) ₂ Cl ₄] 767	Cu

* = decomposition

Infrared spectra:

The important infrared spectra data of ligand and their complexes are given in Table (2). The bands in the region 1618-1578 cm⁻¹ due to $\nu(\text{C}=\text{N})$ vibration azomethine group in the ligand respectively. Fig. (5) Referred to the FTIR spectrum of prepared ligand. These values are increased shifted to lower frequencies (azomethine group) after complexation (17). The bands $\nu(\text{C}=\text{C})$ in region 1514-1480 cm⁻¹ have not changed which remains in the same region in free ligand and after complexation that meant this group didn't coordinated with metal in complexes. New weak bands in the region 415-450 cm⁻¹ were observed in the spectra of metal complexes, which were not appeared in the spectra of ligands due to $\nu(\text{M}-\text{N})$ (18). Table.2 refers to the important bands of the ligand and its complexes. Fig.(6) was referred to FTIR spectrum of copper complexes.

**Fig. (5): Showed FTIR spectrum of Prepared Ligand.**

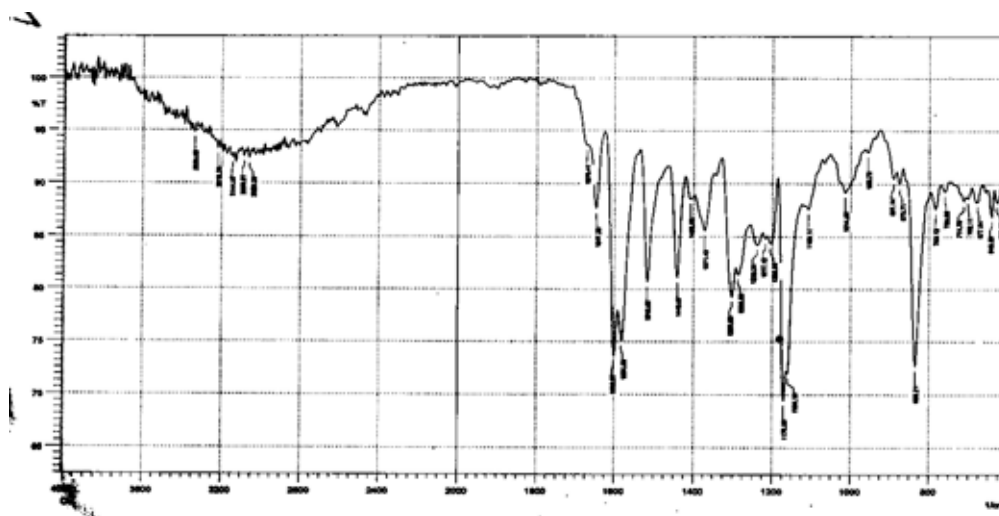


Fig. (6): Showed FTIR spectrum of Copper complex.

Table.2 Showed important bands of the ligand and its complexes

ν (M-N)	ν (M-O)	ν (C=C)	ν (C=N)	ν (H ₂ O)	Molecular formula
...	-----	1514(M)	1587(M)	-----	C ₁₆ H ₁₆ N ₂
422	457	1518	1599		[Co(L) ₂ Cl ₂]
424	451	1517	1599	3419b	[NiL(H ₂ O) ₂]Cl ₂
429	456	1524	1645		[Cu ₂ (L) ₂ Cl ₄]
437	462	1518	1599		[Cd(L) Cl ₂]

B=broad, S=strong, W=weak, M=med

Electronic Spectrum:

Prepared ligand: The electronic spectra of the synthesized ligand showed a strong band at 41680 cm⁻¹ and moderate band at 33315 cm⁻¹ which are attributed to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ respectively.

Cobalt complex: The electronic spectra of Cobalt(II) complex showed two absorption bands at 15490 cm⁻¹ and 23760 cm⁻¹ these were assigned to $4T_{1g}(F) \rightarrow 4A_{2g}(F)$ (ν_2) and $4T_{1g}(F) \rightarrow 4T_{1g}(P)$ (ν_3) transitions respectively. The value of ν_1 was established by theoretical calculation is equal to 9746 cm⁻¹, this value lying in the near infrared zone. Racah's interelectronic repulsion parameters (B) was also calculated by using

relation which was equal to (960.9 cm⁻¹) is lower than the respective B of the free cobalt ion (971 cm⁻¹) indicating delocalization of the metal electrons over molecular orbitals. The value of nephelauxetic effect (β) was established by (B complex / B-ion) ratio which was

equal to (0.98) (19).

The magnetic moment of cobalt(II) has been found to be (4.79 B.M) this value of magnetic moment is higher than the spin only value (3.87 B.M) for their unpaired electrons and may be ascribed to substantial orbital contribution to the magnetic moment value which is applicable of high spin octahedral Cobalt(II) complexes. Fig. (7) is referred to the electronic spectra of the cobalt complex (20).

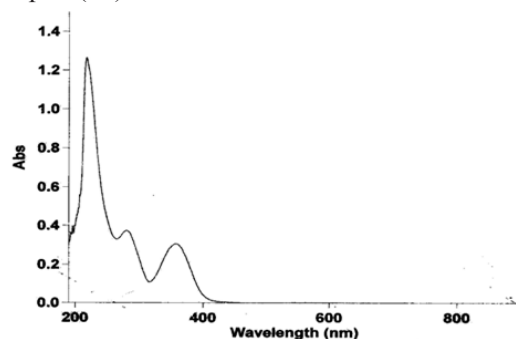


Fig.(7): Showed Uv-visible spectrum of cobalt complex.

Nickel complex: Ni(II) d⁸ (Term 3F) complex is paramagnetic with a room temperature magnetic moment of 2.43 B.M. which is consistent with an octahedral field and correspond to two unpaired electrons as expected for six coordinated spin free Ni(II) species. The magnetic moment for nickel (II) complexes are (2.43 B.M) and. the electronic spectra of this complex showed two bands $d-d$ transitions at 15330 cm⁻¹ and 21200 cm⁻¹ respectively, which may have existence of $3A_{2g}(F) \rightarrow 3T_{1g}(F)(\nu_2)$ and $3A_{2g}(F) \rightarrow 3T_{1g}(P)(\nu_3)$. practical ν_2 and ν_3 values was used to calculate the ν_1

$[3A_{2g}(F) \rightarrow 3T_{2g}(F)]$ position by Lever tables. Also these tables were employed to calculate the ligand field factors, 10 Dq and B that found to be 8516 cm⁻¹ and 732 cm⁻¹ respectively. The calculated B value (732 cm⁻¹) for the complex was lower than the respective B-value of the free nickel ion (1030 cm⁻¹) and the ratio of (B_{complex} / B_{ion}) was showed a value of (0.71). On the basis of spectral bands, an octahedral geometry was proposed for the Ni(II) complex. [19, 20]

Copper complex: The magnetic moment value of copper(II) complex is (0.223 B.M) may express a dimer form in an octahedral structure which might be accrued by coupling of two unpaired electrons on the two copper (II) ions. Electronic spectrum of this complex showed one broad band of (d-d) transitions within the range 14895-15320 cm⁻¹ attributed to the electronic transition ($2E_g \rightarrow 2T_g$) that led to suggest an octahedral structure around the central copper ion (19).

Molar Conductivity measurements:

Molecular conductivity of cobalt, nickel and copper complexes was achieved with concentrations of 10⁻³ M by using of dimethyl sulfoxide as solvent when the cell constant equal to (K = 1). The cobalt and copper record non-electrolytic behavior through the given molar conductivity values where these values indicate the presence of chloride ion within the coordinated sphere (4.3, 25.1) respectively. The nickel complex indicated an electrolyte behavior with a value of (69.7) which indicated the possibility of chloride ion outside the coordinated sphere. The values obtained for the molar conductivity of all the prepared complexes results consistent with the proposed formula for these complexes and are consistent with the results of the other measurements (21).

Photo – Kinetic study of copper complex:

By using integral methods to determine the value of order photo-reaction beside of reaction constant. Applied of first reaction law for three concentration copper complex proved that the photo reaction was from the first order. From the first law, the value of rate constant at for three different concentrations with the constant other conditions such as temperature and pressure gave the same value. To check that the order has not from the other order, the laws of zero, second and third order were applied on the three various concentrations and gave different values of rate constant to give us evidence that the reaction was not from these orders. These calculations were done to enhance that reaction was from the first order. So the equation of the first order reaction was applied and a slope of the plot between time radiation at x-axis and the natural logarithm of the quantity $[a/a-x]$ referred to the value (-K) by which rate constant calculated. Value of rate constant were (0.017) min⁻¹ for the three concentrations (1×10^{-4}) M, (0.5×10^{-4}) M and the concentration (0.1×10^{-4}) M. The plot between the time at the X-axis and concentration changing at the Y-axis of copper complex was achieved. This relationship recorded that concentration followed inverse relationship with the time of photo-degradation reaction, the when the time was increasing the concentration was decreased. Fig.(8). showed the way by which the rate constant was gained with the significance of irradiated time and natural logarithm of (a/a-x), Fig.(9) showed the relationship between the concentration and time of radiation (11).

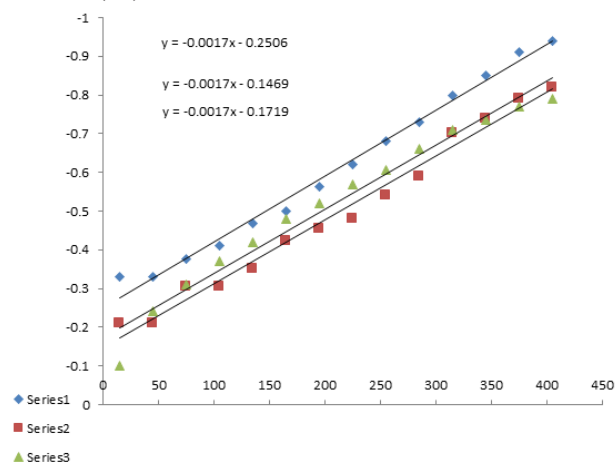


Fig. (8): referred to the relationship between Time on X-axis and $\ln C_t - C_0 / C_0 - C_{\infty}$ on Y-axis.

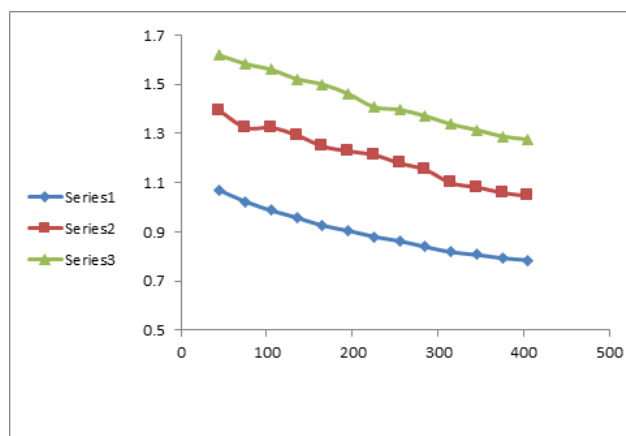


Fig.(9): Showed the relationship between complex concentrations against the radiation time.

Conclusions

The synthesized ligand associated to the selected metal ions(Co+2, Ni+2 and Cu+2) from four sits as a tetradentate ligand .All the geometries of the prepared complexes were octahedral with molar ratio (1:1) ligand to metal except the copper complex was dimer with (2:2) ligand to metal . The photo-kinetic study of the copper complex showed that the reaction was first order reaction in the terms of complex concentration with the neglected of solvent amount ,also the copper complex has photo sensitivity toward ultra-violate spectroscopy.

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Conflict of Interest: Authors declare that, there is no conflict of interest regarding the publication of this paper.

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Effect of Aqueous Extract of Clove *Syzygium aromaticum* Against Pathogenic Fungus *Alternaria* spp. Isolated From Skin Infections

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Abstract

The Aim of this Study are to evaluate the potential antifungal activity of clove aqueous extracts against *Alternaria* spp. as well as, the smallest concentration capable of inhibiting or preventing their growth, during the period from April 2017 to April 2018, cross sectional study of 48 patients done with 26 of them established diagnosis with *A.spp*, from them 5 females 19.3% and 21 males 80.7% with ages range of 17-55 years, study was done in Al-Karama Teaching Hospital, *Alternaria* species isolated from skin lesion taken from patient in dermatology department in the hospital, the lesion was excised and cultured on potato dextrose agars (PDA) and Sabouraud dextrose agar (SDA) colonies developed, effect of clove aqueous extract with three concentrations (5%, 10% and 20%) was assessed by agar diffusion plate method, The radial growth of colonies has been measured and on the basis of these values the percentage of inhibition of colony growth has been calculated, the outcome displayed as tables and figures, the antifungal activities of aqueous extract of clove against *Alternaria* spp. Was assessed by measuring diameter of colonies in PDA and, SDA which found to be 4cm in PDA with 44.4% growth inhibition percentage, and colony diameter was 5cm in SDA at 5% concentration with 55.5% growth inhibition percentage, comparing with control which show 9 cm colony diameter in both PDA, and SDA, While no growth detected in both SDA and PDA at 10% and 20% concentration comparing with control with 100% growth inhibition percentage. Our result shows growth dependent effect on extract concentrations, with decrease growth by increase concentration of extract. In conclusions the findings of the present study indicate that the clove aqueous extract in different concentration has interesting potential effect as a natural therapeutic option against fungi that are pathogenic to humans.

Key words : clove extract, *Alternaria* spp, *Syzygium aromaticum*, Antifungal, Eugenol.

Introduction

Cloves *Syzygium aromaticum* is widely used an aromatic herb that has many useful medicinal purposes as a natural anti-fungal agent (1) with effectively inhibit the growth of wide range of microorganism with fewer side effect to human than synthetic agent with strong antifungal activity against opportunistic fungal pathogens (2).

So the cloves has been used in a variety of health conditions recently approved the use as a topical antiseptic and anesthetic, and it's one of the highest sources of manganese which is vital for metabolism, containing

Magnesium, calcium responsible for its strong appearances in clove, as well as many phytonutrients that enhance the immune system (3).

The incidence of fungal infections has been increased, In spite of introduction of new antifungal drugs, which still limited in number. The majority of clinically used antifungal have element of toxicity, in addition to their cost, there is great demand for new antifungal agents less toxic than those already in use (4), less environmental effects and wide public acceptance (5), Clove has been widely investigated due to its popularity, availability, and high essential oil content (6), antimicrobial properties of these plants are well

documented against bacteria, fungi and yeasts (7).

The leaves of the clove are leathery textured and are covered with many tiny depressions. And The part of the clove that is used of the flower buds (8). While the mechanism of action of clove depend on its essential ingredient which responsible for its antifungal activity including eugenol clove bud that is used in traditional medicine have high content of eugenol 87% which cause a considerable reduction in the quantity of ergosterol, which is the major component of the fungal cell membrane, being responsible for maintaining cell function and integrity (9), which is thought to be the mechanism of antifungal action of this plant extract(10,11), same mechanism of action by which azole antifungal drugs inhibit fungal cell growth by disruption of normal sterol biosynthetic pathways, leading to a reduction in ergosterol biosynthesis (12), besides eugenol 87%, β -caryophyllene 4–21 %, eugenyl acetate 0.5– 21 %, smaller amounts of α -humulene along with trace amounts <1 % of 25–35 other constituents (13). But *Alternaria* spp. is widely distributed in nature and this species are among the most common fungi on the phyllosphere regrowth occurs as thick grey wooly colonies with a dark undersurface on Sabaroud dextrose agar (14), and most of the human infections occur due to traumatic inoculation and cutaneous infections (15). *Alternaria* may be increased due to high corticosteroid drug usage which increase fragility of skin leading to increase involvement (16,17), with wide spectrum of infection ranging from non-invasive colonization to systemic infection, the infection usually appear as skin erythema in average age of 54 y, the prevalence in men was 65% comparing to 35% in women, the reason for this probably a higher rate of exposure to external effects (18,19). Therefore the aim of this work were to evaluate the antifungal activity of clove aqueous extracts against *Alternaria* spp. as well, the smallest concentration capable of inhibiting or preventing their growth.

Materials and Method

1. Plant materials

The dried flower buds of clove plant used in this study were purchased from local market.

2. Preparation of extract:

Aqueous Extract was prepared by use clove buds 200 g were mixed with 500 ml Distil water and heated

for 5-10 min at 60 °C then filtered by double layered muslin cloth resulting in aqueous extract kept under sterilized conditions.

3. Preparation of cultured media :-

39 g from Potato Dextrose Agar (PDA) mixed with 1000 ml distal water and 65g from Sabroid Dextrose Agar (SDA) mixed with 1000 ml distal water then both sterilized by autoclave at 121 °C, and 15 pawnd pressure for each inch for 15 min (20).

After that 5 ml from aqueous extract completed to 100ml by adding PDA to get extract with 5% concentration, 10 ml from aqueous extract completed to 100ml by adding PDA to get extract with 10% concentration and 20 ml from aqueous extract completed to 100ml by adding PDA to get extract with 20% concentration under sterilized, same procedure done by use SDA to get extract with same concentration mentioned above (21).

4. Fungal strain: During the period from April 2017 to April 2018, cross sectional study of 48 patients done with 26 of them established diagnosis with *Alternaria*, from them 5 females 19.3% and 21 males 80.7% with ages range of 17-55 years, the study was done in Al-karama Teaching Hospital.

Also *Alternaria* species isolated from skin lesion as shown in figure(1) taken from patient in dermatology department in the hospital, the lesion was excised and cultured on potato dextrose agar (PDA), and Sabroid dextrose agar (SDA) at 25 °C, after 10 days, flat colonies developed which is black at 25 °C and covert to off-white at 37 °C.

Microscopic examination in slide cultures revealed short chains composed of two to five celled dark smooth walled conidia, with oblique to transverse septa, arising terminally with lateral branches

5. Antifungal activity of extract:

Effect of clove aqueous extract in PDA and SDA with three concentrations 5%, 10 % and 20% were assessed by agar diffusion plate method, the radial growth of colonies has been measured and on the basis of these values the percentage of inhibition of colony growth has been calculated by formula given by (22).

$$\text{Percent inhibition (\%)} = \frac{\text{ge-gt}}{\text{ge}} \times 100$$

Where, gc is the growth in control plates, gt is the growth in treated plates

6. Effect of plant extract on the weight of dried *Alternaria* spp.

Mixed 100 ml of PDB with above mentioned concentrations of extract each separated in clean flasks then in each flask 0.5 cm from *Alternaria* spp. colony is inoculated and kept in incubator for 10 days in 25°C, after that mycelium taken and dried for 48 h and at 70°C, then the weight of *Alternaria* spp. measured.

Findings

The antifungal activities of aqueous extract of clove against *Alternaria* spp. were assessed by measuring diameter of colonies in PDA and SDA as shown in table (1)

Table (1): Show growth diameter in control and extract in different concentration in both PDA and SDA

Treated aqueous extract	Diameter at 5% cm	Diameter at 10%	Diameter at 20%	Diameter in control cm
in PDA	4	Zero	Zero	9
in SDA	5	Zero	Zero	9

which found to be 4 cm in PDA as shown in figure (2) with 44.4% growth inhibition percentage and colony diameter was 5 cm in SDA as shown in figure (3) at 5% concentration with 55.5% growth inhibition percentage as shown in figure (4) comparing with control which show 9 cm colony diameter in both PDA and SDA. While no growth detected in both SDA and PDA at 10% and 20% concentration comparing with control with 100% growth inhibition percentage, as shown in table (2)

Table (2): Show growth inhibition percentage in different extract concentration in PDA and SDA

Concentration %	Growth inhibition percentage in PDA extract %	Growth inhibition percentage in SDA extract %
5	44.4	55.5
10	100	100
20	100	100

these results show growth dependent effect on extract concentrations, with decreasing growth by increasing concentration of extract and according to the effect of extract on the weight of *Alternaria* spp. Also we found that the dry weight of the control fungi + PDB without extract are 0.159 g and dry weight of fungi + PDB with extract at 5% concentration are 0.07 g while at concentration of 10%, 20% no growth was noticed.

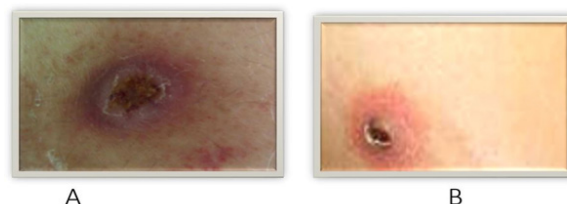


Figure (1): Skin lesion of *Alternaria* spp. taken from medial surface of arm in (A) and lateral surface of leg in (B)

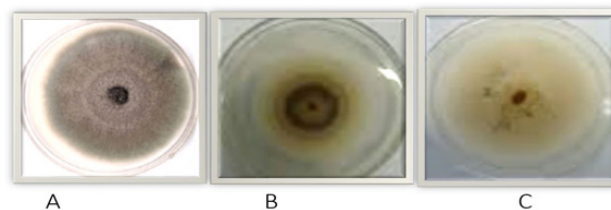


Figure (2) Effect of aqueous extract of clove in PDA comparing with control

(A) control (B) PDA+extract at 5% concentration (C) PDA+extract at 10% concentration

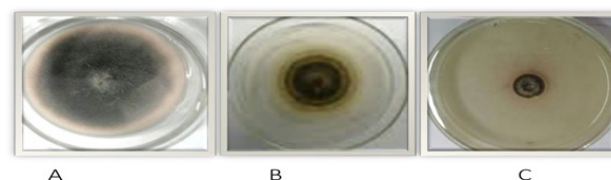


Figure (3): Effect of aqueous extract of clove in SDA comparing with control

(A) Control (B) PDA+extract at 5% concentration (C) PDA+extract at 10% concentration

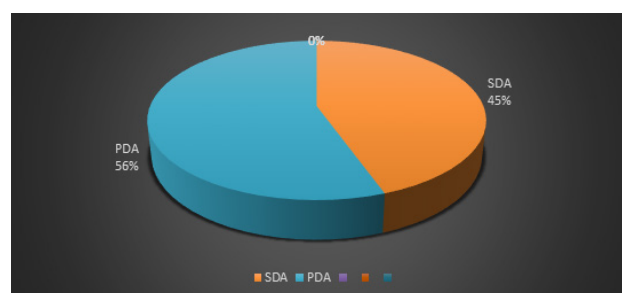


Figure (4): Distribution of growth inhibition percentage in PDA and SDA in this study

This study show the antifungal activity of clove extracts against *Altrnariasp* and decrease of mycelium growth started at the lowest concentration used 5 % with decrease colony diameter from 9 cm in control to 4cm in PDA and 5 cm diameter in SDA with comparable result even by use different media and growth inhibitions successively increased with the increasing of concentration with complete inhibition of growth at concentration of 10 % and 20%, the result of this study are in agreement with previous study conducted by (23), who evaluated the antifungal activity of clove extract against some fungi and they reported that all fungal species in his study were inhibited by the extract, also the main chemical components of the clove are eugenol, with broad spectrum of activity against a variety of pathogenic yeasts and filamentous fungi as shown by (24,25,26).

Limitations

The limitations of this study are summarized by difficulty of getting samples of affected cases, just by specialist which take a lot of effort and cost, in addition to that most affected people lived in rural area, so connection with them a little bit is not easy.

Conclusion

The findings of the present study indicate that the clove aqueous extract in different concentration has interesting potential effect as a natural therapeutic option against fungi that are pathogenic to human.

Conflict of Interest : None

Source of Funding : Self

Ethical Clearance: All samples taken after the patients' approval.

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Effect of Oxygenated Water Alone or with Vaccines and Antibiotics on Immune Response Against Viral Diseases and Intestinal Microflora Community in Broiler Chickens

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Abstract

This study was conducted to investigate the effect of providing oxygenated water with or without vaccines and antibiotic on immune response against Newcastle disease (ND), Infectious Bronchitis disease (IB), Bursal disease (BD), total aerobic bacteria, coliform bacteria and Lactobacilli bacteria in broiler chicks. A total of 160 broiler chicks (Ross 308) one day old were randomly distributed to 4 groups each with 4 replicates (10 birds/replicate) as follows: group 1 (C-) tap water without vaccines and antibiotic, group 2 (C+) tap water with vaccines and antibiotic, group 3 (OW-) oxygenated water without vaccines and antibiotic, group 4 (OW+) oxygenated water with vaccines and antibiotic. Blood samples were collected two times on days 21 and 42 of age to measure antibody titers against ND, IB and BD diseases. Microbial count for total aerobic bacteria, coliform bacteria and lactobacilli bacteria were evaluated at the end of the study (42 days old). The results indicated that the antibody titers against ND, IB, and BD disease were significantly higher ($p < 0.05$) in group OW+ (3903 ± 80.44) and (4731.9 ± 71.6) followed by group C+ (2123 ± 241.1) and (36101 ± 4.7), group OW- (1928 ± 72.34) and (2128 ± 32.7) and finally group C- (924 ± 11.2) and (605 ± 10.3) for the age groups 21 days and 42 days respectively. There were no differences among experimental groups in total aerobic bacteria. The lactobacilli bacteria significantly ($p < 0.05$) increased in the different experimental groups as follows: OW+ (7.01 ± 0.33), OW- (6.01 ± 0.52), C- (5.63 ± 0.31), while the coliform bacteria values were significantly lower ($p < 0.05$) in OW+, C+, OW- and C- and they were (4.74 ± 0.33), (5.67 ± 0.81), (6.7 ± 40.46) and (7.09 ± 0.39) respectively. These results indicate that providing oxygenated water with vaccines and antibodies may be recommended as a cofactor to improve immune response against common viral diseases and pathogenic bacteria in broilers.

Key words: Broiler chickens, Oxygenated water, Vaccines, Antibiotics

Introduction

There are many factors that affect the poultry performance, including nutrition, management, environmental conditions, and breeding system, but water quality may be the most important and the least expensive [1]. Water, constituting 60-85% of bird body weight, is found in all tissues and cells. It plays a vital role in many metabolic processes like regulation of body temperature, digestion, translocation and absorption of feeds, hydrolysis of protein, fat, and carbohydrates, sight and hearing functions, respiration

and perspiration [2]. Thus, the use of water with suitable physical, chemical and microbiological properties has substantial importance in the poultry industry. Among these goodness criteria, level of dissolved oxygen is also important with pH and temperature [3]. Many studies have indicated that oxygenated water has advantageous biological effects. Medical research in the last century has shown the therapeutic effects of oxygenated water for various diseases such as obesity, liver dystrophy and accelerated alcohol detoxification [4], [5], [6]. Recent studies have shown that drinking oxygenated water has a role in reducing the blood glucose levels in patients

with diabetes [7]. Giving oxygenated water to old females mouse for 22 weeks results in a higher weight gain and lower amount of feed intake, as high oxygen concentrations lead to an enhanced rate of oxygen absorption by the body, resulting in increased glycolysis and/or mitochondrial protein synthesis[8].

There were some studies regarding using the oxygenated water in poultry, a positive relationship between the level of dissolved oxygen in drinking water and broiler performance, subsequent studies supported this finding and reported the enriched water with oxygen enhance feed conversion and growth performance in broilers[9],[3]. Other studies were done in this area indicated that oxygenated water has immune-enhancing effects in broiler chickens and Continuous drinking oxygenated water markedly alleviated symptoms and increased survivability against *S. gallinarum* in experimentally infected broiler chicks[10]. Drinking oxygenated water improve growth performance by increasing immunoglobulin's mainly IgG and IgM in broiler chickens[11].

Recently, there are increasing attention to improving immune status to commercial birds by using a natural agents to stimulate immunity system as an alternative method to reduce the use of veterinary drugs and vaccines, thus and based on the immunological effects of drinking oxygenated water as mentioned above, we carried out this study to evaluate the effect of oxygenated water on immune response against some conventional diseases such as Newcastle disease (ND), Infectious Bronchitis disease (IB), Bursal disease (BD) and intestinal microflora as a primary step to use eco - friendly method to reduce the dependence on veterinary drugs and vaccines.

Materials and Method

Experiment Design:

This experiment was conducted to explore the effect of drinking oxygenated water on the immune response against some conventional diseases (Newcastle, Infectious Bronchitis and Bursal diseases) and the intestinal microflora of broiler chickens from hatching to 42 days of age. A total of 160 unsexed day-old broiler chicks (Ross 308) obtained from a commercial hatchery were randomly allocated to 4 groups with 4 replicates / group (10 birds/replicate) as follow:

Group 1: C-, the chicks received normal tap water and fed a diet did not contain antibiotic and not vaccinated against ND, IB and BD diseases.

Group 2: C +, the chicks received normal tap water and fed a diet contain antibiotic and vaccinated against ND, IB and BD diseases..

Group 3: OW-, The chicks received oxygenated water and fed a diet did not contain antibiotic and not vaccinated against ND, IB and BD diseases..

Group 4: OW +, the chicks received oxygenated water and fed a diet contain antibiotic and vaccinated against ND, IB and diseases.

Birds Housing and Management:

The chicks were reared in the floor pens (1* 1.5 m²) covered with wood shavings, the experimental room temperature was 35 ± 1.1°C for the first 3 days and reduced to 32°C until the end of 1st week, this was subsequently reduced by 3 - 5 °C per week until 23°C on day 21 and maintained to the end of the study, the illumination program was 23 h light vs. 1 h darkness the chicks were fed two phases of diets, a starter diet (1 - 21 days) contained 21.14 % crude protein and 3050 ME, Kcal/kg and finisher diet (22 - 42 days) contained 19.2% crude protein and 3200 ME, Kcal. The diet and water offered ad libitum throughout experiment period and Vaccination Program conducted according to[12]:

Oxygenated water preparation:

Two tanks (500 L) were placed in the experiment room and filled with normal drinking water, the Enrichment of water with dissolved oxygen in one of them was via an oxygen cylinder for 3 hours / 3 times/day. The dissolved oxygen concentration in drinking water was measured by HORIBA apparatus (USA) where it was 16, 23 mg/l in oxygenated water vs. 5, 5 mg/l in non-oxygenated water throughout the experiment period.

Serum Antibody Titer:

To estimate antibodies titer against ND. IB and BD disease, 3 birds were randomly chosen from each replicate to collect blood samples. A total of 48 blood samples (12 sample/ group) were taken from the wing vein at 21 and 42 days of age, The serum was separated

by centrifugation (3000 RPM 15 minutes) and antibodies titration against these diseases were measured by the ELISA method using standard kits (ProFLOK® PLUS Synbiotics Corporation –San Diego/USA). Intestinal microbial enumeration according to [13].

Statistical analysis:

Data were subjected to analysis of variance [14], and significant means were separated by [15].

Findings and Discussion

Table 1: Antibody titrations against Newcastle (ND), infections (IB) and burat (BD) diseases by ELISA method on 21 and 42 days age.

group					
Age (day)	Virus	C-	C+	OW-	OW+
21	ND	468 ± 37.5 D	1383 ± 24.4 B	1231 ± 20.6 C	2111 ± 52 A
	IB	217 ± 16.4 C	1602 ± 41.2 A	1252 ± 37.9 B	1627 ± 23.5 A
	BD	924 ± 11.2 D	2123 ± 241.1 B	1928 ± 72.34 C	3903 ± 80.44 A
42	ND	184 ± 6.78 D	3242 ± 152.6 B	1201 ± 103.8 C	4242 ± 172.1 A
	IB	115 ± 3.3 D	3610 ± 14.7 B	2128 ± 32.7 C	4731.9 ± 71.6 A
	BD	605 ± 10.3 D	1970 ± 83.6 B	1085 ± 51.6 C	2559 ± 131.5 A

C - : Tap water without vaccines and antibiotic. C +: tap water with vaccines and antibiotic, OW - : oxygenated water without vaccines and antibiotic, OW +: oxygenated water with vaccines and antibiotic. Mean ± standard error. Different letters in each row indicate significant differences ($p \leq 0.05$)

Table 1 shows significant differences ($p \leq 0.05$) of antibodies titer among experimental groups against ND, IB, and BD viruses. The highest values were in OW + followed by C + and then OW - and finally C - at 21 and 42 days old. OW + (3903 ± 80.44) and (4731.9 ± 71.6) followed by group C + (2123 ± 241.1) and (3610 ± 14.7), group OW - (1928 ± 72.34) and (2128 ± 32.7) and finally

group C - (924 ± 11.2) and (605 ± 10.3) for the age group 21 days and 42 days respectively. The potential reasons for these results can be elucidated as expected as follows, the lowest values for antibodies titer were in group C - compared to other groups to not enhance their immunity against these diseases by vaccines, on the other hand, the modern broilers strains characterized by their rapid growth, which requires more oxygen [16]. But their cardiopulmonary system very similar to the old broilers strains which makes it does not always meet the oxygen demands necessary for rapid growth [17].

If the cardiopulmonary system of chicken grows less rapidly than the rest of the body, hypoxia

could result [18]. Low oxygen levels (hypoxia) can result in increase in reactive oxygen species (ROS) production from the mitochondria in lymphocytes [19], reducing the mitochondria's ability to generate ATP [20] and intracellular anti-oxidants become less available [21]. The low levels of antioxidants lead to reduce IL-2 production, a cytokine that is required for prolonged lymphocytes survival and growth.. We can also conclude

from the data in this table that oxygenated water is not a substitute for vaccines because the antibodies titer in group OW - were significantly less than that of group C + but could be a helpful factor in enhancing body immunity through its synergistic action with vaccines and results in group OW + Supports this conclusion. This group recorded the highest values in the antibodies titer compared with the other groups.

Table 2: Effect of oxygenated water on microbial population of broiler intestine.

Microbial parameters	Group			
	C-	C+	OW-	OW+
Total aerobic count	7.24 ± 0.66 A	7.40 ± 0.67 A	7.71 ± 0.49 A	7.89 ± 0.45 A
Coli form count	7.09 ± 0.39 A	5.67 ± 0.81 C	6.74 ± 0.46 B	4.74 ± 0.33 D
Lacto. B. count	5.63 ± 0.31 C	6.17 ± 0.61 B	6.01 ± 0.52 B	7.01 ± 0.33 A

C - : Tap water without vaccines and antibiotic.
C + : tap water with vaccines and antibiotic , OW - : oxygenated water without vaccines and antibiotic , OW + : oxygenated water with vaccines and antibiotic . Mean ± standard error. . Different letters in each row indicate significant differences ($p \leq 0.05$) .

The results in table 2 showed no significant differences were observed in total aerobic bacterial count among different groups. Comparison of averages obtained from the experiment showed that group OW+ had the most influence ($p < 0.05$) on coliform (7.09±0.39) and lactobacilli bacteria (7.01±0.33) compared with the other groups . Also We note from Table 2 that the addition of the antibiotic to the diet (C+) (7.40±0.67) and enriched water with dissolved oxygen (OW-) (7.7±10.49) has a significant effect ($p < 0.05$) to improve intestinal microbial community. The antibiotic effect was significantly ($p < 0.05$) greater than the oxygenated water in reducing the number of coliform bacteria While no significant differences between these two groups on lactobacilli bacteria.

The microbial community in birds which drank oxygenated water in the group OW - was indicative of the dominance ($p < 0.05$) lactobacilli bacteria and declines a proliferation of coliform bacteria. It appears

that the synergistic effect of oxygenated water and antibiotic played an important role in achieving the best results regarding intestine microbial community in the oW+ group compared with other groups.

Conclusion

The inhibitory effect of both oxygenated water and antibiotic against pathogenic bacteria (coliform bacteria) will be allowed to multiply and increase the count of the beneficial bacteria such as lactobacilli in the intestine and inhibit colonization of the pathogenic bacteria because of the lactobacilli bacteria produce lactic acid beneficial to them and deleterious to the coliform bacteria and other pathogenic.

Recommendation

We need more studies regarding the appropriate levels of dissolved oxygen in the water and its impact on the productive and immunological aspects of broilers chicken and other types of domestic birds.

Conflict of Interest- Non

Funding – Self

Ethical Clearance- With agreement

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Urogenital Infection and Male Infertility: The Presence of Leukocyte in Semen is Associated with Decrease Fertilization Potential of Human Spermatozoa in Infertile Patients affected with Leukocytospermia

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Abstract

This study was designed to evaluate the effects of leukocytes contamination determined by peroxidase-positive leukocytes on semen characteristic of leukocytospermic subjects. Fifty (25 leukocytospermic vs. 25 non leukocytospermic) infertile couples collected from Al-Hussein Teaching Hospital/ Thi-qar Health Directorate enrolled in this study and semen samples were analyzed before and after in vitro sperm preparation technique. Furthermore, sperm concentration, sperm motility, progressive sperm motility, normal sperm morphology, and seminal leukocytes counts were evaluated according to standard WHO criteria (2010). For preparation technique, sperm prepared and incubated for 30 minute in 5% CO₂ at 37°C. The results of present study shows that leukocytospermic men significantly ($P < 0.001$) decreased and differences sperm functions compared with non leukocytospermic men may be due to reactive oxygen species generation by leukocytes. It was concluded that the increase seminal leukocyte counts lead to impaired sperm parameters and decrease fertilizing ability of human spermatozoa and sperm function improved and augmented after sperm processing technique. Further studies are recommended to assess the harmful outcomes of ROS on DNA damage and embryo quality after intracytoplasmic sperm injection (ICSI-ET).

Key words: Leukocytospermia, Fertilization, Infertile Patients, peroxidase-positive leukocytes

Introduction

In European countries nearly 10-15% of couples are afflicted by infertility. In 60% of a male factor is implicated [1]. The distribution of etiological factors for male infertility reveals that apparently 30% of patients suffer from idiopathic infertility [2]. Acute or chronic infection of the genitourinary tract may be a contributing role in male factor infertility [3]. Infectious processes impair fertility by different mechanism including deterioration of spermatogenesis, impairment of sperm function, and obstruction of the seminal tract [4]. Leukocytospermia is used to designate abnormally high concentrations of the white blood cells (WBCs). The prevalence of leukocytospermia in male infertility patients varies from 20%-40% depending on patients

population [5]. By peroxidase positive leukocytospermia staining only polymorphonuclear leukocytes (PMNL) can be detected. Lowering Leukocytospermia threshold value to 5×10^5 PMNL/ ml of semen provide comparable data using 1×10^6 total WBCs/ ml of semen [6]. There are two main sources of ROS in semen: leukocytes and immature spermatozoa of these, leukocytes are considered to be main source [7]. Leukocytes, particularly neutrophils and macrophages, have been associated with excessive ROS production that ultimately leads to sperm dysfunction [8]. Spermatozoa produce ROS mainly when a defect occurs during spermiogenesis that results in retention of cytoplasmic droplets; the two main sites of ROS production are the mitochondrion and sperm plasma membrane. The mitochondrion is the

powerhouse of respiration. Hence, it is the major site of its, which is produced through nicotinamide adenine dinucleotide-dependent oxido-reductase pathway^[9].

In the background of human reproduction, a balance called oxidative stress status (OSS) normally exists between ROS production and antioxidant scavenging system in male reproductive tract^[10]. Small physiological levels of ROS are essential for regulation of normal sperm functions such as sperm capacitation, acrosome reaction, and sperm-oocyte fusion^[11]. Though, production of excessive amounts of ROS in semen especially during leukocytospermia can overcome antioxidant defence mechanisms of spermatozoa and seminal plasma resulting in oxidative stress^[12]. However, Human ejaculate consists of different types of cells such as mature and immature spermatozoa, round cells from different stages of spermatogenic process, leukocytes and epithelial cells. Spermatozoa are also particularly susceptible to the damage induced by excessive ROS because their plasma membranes contain large quantities of polyunsaturated fatty acids (PUFA), which readily experience lipid peroxidation by ROS, resulting in a loss of membrane integrity^[13]. During genital tract infection, the presence of leukocytes in semen has been associated with decreased sperm motility and fertilization capacity. This impasse may be partially due to the different techniques used to determine leukocyte concentration in semen as well as the lack of agreement on the lower leukocyte concentration responsible for sperm damage^[14]. Sperm function may also be indirectly affected by an infection stimulating the presence of ROS in the prostate gland and seminal vesicles^[15]. Multiple hypotheses discuss male GTI and their relationship with ROS. Specifically, leukocytes stimulate human spermatozoa to produce ROS. The mechanisms responsible for such stimulation are unknown, but may include the direct contact of sperm and leukocytes or may be regulated by leukocyte release of soluble products^[16].

Materials and Method

Patients and semen collection

Fifty infertile males were enrolled in this study and semen samples were obtained from Thi-Qar health directorate/ Al-Hussein Teaching Hospital. The mean age of infertile subjects was 33.42 ± 0.56 years old with range from 22-59 years and duration of infertility was 9.64 ± 0.32 years with range from 4-18 years. The

semen samples were collected by masturbation after 3-5 days abstinence and allow liquefying at 37°C in 5% CO₂ for 30 minutes and evaluated before and after *in vitro* sperm preparation. Sperm functions tests were performed manually including semen samples with sperm concentration $\geq 20 \times 10^6/\text{ml}$, sperm motility $\geq 50\%$, progressive sperm motility $\geq 50\%$, normal sperm morphology $\geq 30\%$, and seminal WBC counts $\leq 1 \times 10^6/\text{ml}$ were evaluated according to WHO guidelines. The number of peroxidase-positive leukocytes in semen was determined using Endtz test. However, leukocytospermic ($>1 \times 10^6$ leukocytes/ml) (n=25) and non-leukocytospermic ($\leq 1 \times 10^6$ leukocytes/ml) (n = 25).

In vitro Semen processing techniques

Conventional layering technique

The semen was prepared by using 1ml of prepared culture medium was added to test tube, and then 1ml of liquefied semen was layered beneath a culture medium. After incubation for 30 minute in 5% CO₂ at 37°C, 10μl. of the mixture was aspirated by pasture pipette and examined under light microscope at 400X magnification for assessment parameters of sperm functions in infertile patients.

Peroxidase-positive leukocytes staining

A 20 μl volume of liquefied semen specimen was mixed together with 20 μl of phosphate buffered saline (PBS; pH 7.0) and 40 μl of benzidine solution. The mixed solution was allowed to settle at room temperature for 5 minute. Peroxidase-positive leukocytes staining brown were counted.

Statistical analysis

Statistical analysis was performed with the SPSS version 15.00. The data analysis was done using paired sample t-test to assess statistical differences. Mean and standard error of mean (S.E.M) obtained from crude data to compare between seminal fluid analysis parameters. P-value < 0.05 was used as a level of statistically significant.

Findings

After sperm processing using culture medium prepare with simple conventional technique, sperm concentration and leukocyte counts were significantly

($P < 0.001$) decreased as compared to pre-processing in leukocytospermic and non leukocytospermic samples, while sperm motility, progressive sperm motility, normal sperm morphology, were significantly ($P < 0.001$) increased in leukocytospermic and non leukocytospermic samples post-*in vitro* sperm processing as compared to pre-processing but the best results appears clearly in leukocytospermic samples as a results of the selection of sperm preparation methods depend on quality of the ejaculates. The selection of sperm preparation methods depend on quality of ejaculates. In addition, the ejaculates with ROS production by spermatozoa and leukocytes should not be separated by centrifugation method due to severely damage the spermatozoa [17]. Also, Gellert-Mortimer *et al.* [18], suggested that when semen samples prepared by centrifugation technique, functional spermatozoa can come into close cell-to-cell contact with defective sperm, leukocytes, and cell debris contained by centrifugation force causing massive oxidative damages of sperm plasma membrane via produce very high levels of ROS by pelleting of the semen with impairment of sperm functions and decrease in percentage of normally chromatin-condensed spermatozoa [19]. It was noticed that the problem caused by ROS can resolve by performed directly from the liquefied semen underneath an overlay of culture medium and aspirate directly from the interface region with total number of spermatozoa recovered [20]. However, Makkar *et al.* [21], certified that a conventional layering directly from semen into a hyaluronic acid significantly higher percentage of motile spermatozoa and pregnancy rate in a clinical IVF or IUI program may be as a result enhanced sperm quality by decreased release of lymphokines and cytokines after sperm preparation [22]. In contrast, it was assessed that centrifugation force adversely affects sperm motility and impairment of acrosome reaction, sperm membrane, and nuclear maturity rates in men with abnormal and normal semen analyses in comparison to density gradient

centrifugation [23]. The sperm functions parameters results after direct swim-up technique strongly correlated to predict embryo cleavage. Furthermore, it was reported that common laboratory factors like centrifugation, washing, and temperature fluctuation of human spermatozoa both positively and negatively due to direct influence of the laboratory interventions on the cytoskeletal assemblies of sperm [24]. Undoubtedly, the spermatozoa selected by layering technique enhanced sperm penetration results in zona free hamster egg by sperm penetration assay [25]. The plasma membrane of spermatozoa is particularly susceptible to ROS induced damage because of its high content of polyunsaturated fatty acids (PUFA), Sperm cytoplasmic volume is very low and its cytoplasm contains only low concentrations of free radical scavenging enzymes [26]. However, oxidative stress (OS) plays an important role in human reproduction and arises as a consequence of excessive ROS production and/or impaired antioxidant defence mechanisms. Also, free radicals may have beneficial or detrimental effects on sperm functions, depending on their nature and concentration [27]. The pathological levels of ROS detected in the semen of infertile men are probably caused by increased ROS production rather than reduced antioxidant capacity of seminal plasma. The presence of excess residual cytoplasm (or cytoplasmic droplets) is a major determinant of ROS generation by these defective spermatozoa [28]. Oxidative stress-mediated damage to the sperm plasma membrane may account for defective sperm function observed in a high proportion of infertility patients. Oxidative stress-induced DNA damage may accelerate the process of germ cell apoptosis leading to the decline in sperm counts [29]. A spermatozoon was the first cell type suggested to generate highly reactive oxygen derived free radicals. Elevated ROS level can indicate either semen contamination by leukocytes (especially granulocytes) or the occurrence of defective and/or immature spermatozoa [30].

Table (1): The effect of sperm activation techniques prepared by conventional layering method on sperm functions and leukocytes concentration in leukocytospermic and non leukocytospermic patients determined by Peroxidase-positive leukocytes.

Parameters	Conventional layering technique (SPT)			
	Leukocytospermic (n=25) >1 x 10 ⁶ leukocytes/ml		Non leukocytospermic(n=25) ≤1 x 10 ⁶ leukocytes/ml	
	Pre-Prepared	Post-prepared	Pre-treated	Post-treated
Sperm Concentration (×10 ⁶ sperm/ml)	42.23±5.33	20.45±2.55 ^a	49.75±3.32	23.38±2.1 ⁰ ^a
Sperm Motility (%)	57.12±2.50	71.52±2.13 ^a	54.15±3.22	82.15±2.14 ^a
Progressive sperm Motility (%)	33.60±1.53	51.65±2.23 ^a	45.10±1.90	70.35±2.23 ^a
Normal Sperm Morphology (%)	35.52±2.21	68.15±2.09 ^a	52.52±2.50	85.20±2.08 ^a

Values are Mean ± S.E.M

a: means a highly significance (P<0.001) different from pre-preparation.

No. of infertile patients=50 for conventional layering technique

Mean of age ± S.E.M for infertile subjects prepare with layering technique (33.42 ± 0.56 years)

Values are Mean ± S.E.M

Conclusions

It was concluded from the present study that the increase seminal leukocyte counts lead to impaired sperm parameters and decrease fertilizing ability of human spermatozoa and sperm function improved and augmented after sperm processing technique. Further studies are recommended to assess the harmful outcomes of ROS on DNA damage and embryo quality after intracytoplasmic sperm injection.

Conflict of Interest: There is no conflict of interest during the collection of semen samples of research from infertility patients, but things were easy and simple and cooperate with us all to reverse the search and get good results through which can solve a real problem in Iraqi

society is the problem of male infertility patients with urinary tract infections.

Sources of Funding: The self-help of the researchers was based on the research, where all the materials, the culture media and the materials needed for the test were purchased for maintenance on special expenses for authors.

Ethical Clearance: It is within the moral conclusion that the secrets of the patients are kept private by not informing others about the person and his family as things should be within the scope of scientific research only as well as the researcher should be honest and frank with the patients by giving them the result with all honesty and transparency and work with all circumstance Sincerity in order to obtain a result can be a reason to solve their problem.

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Study of Some Microbial Contents of Crisps & Cakes Specimens which Available in Supermarkets

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Summery

A 260 specimens of crisps & cakes were examined, represented by 26 kinds of crisps and 26 kinds of cakes. Each one repeated five times. They cultured on Nutrient agar and brain heart infusion agar, after the incubator period 8 different types of Bacteria were diagnosed. In crisps, the more frequency was *Bacillus* sp. Then *E. coli*, *candida*, *Klebsiella*, *Diplococcus* and others. Some Specimens without any microbes. Sixteen types of cakes were polluted with microbes. Also *Bacillus* sp. took the largest numbers then *E. coli*, *Staph. sp.* *Strep. sp.* others respectively. *Bacillus* were more prevalence and frequency in both kinds of food, it may be for its availability in air, its tolerance to hard unsuitable conditions, formation of resistance spores. The Turkish cakes was suitable for eating, salt & vinegar crisps were empty of any microbe's kind.

Keywords :- microbes, contamination, cakes, crisps

Introduction

There are many different snacks in supermarkets; some are canned, dried, fresh and others. They offer change according to their types, shapes, tastes etc. children tend to use crisps, cakes, chocolates, juices, biscuits and others as the main food for them. The food factories put special flavours and colors (1), conservatives to maintain their product from pollution and to be better. The ways of their storage till sailings are differs from company to others, for this reasons it may provide a suitable environment for some microbes to grow, reproduce and cause some disease (2).

Some microbial food poisoning were recorded as mentioned by that the bloody diarrhea was resulted from the first time of *E. coli* contamination in hamburger since 1982 (3). Some scientists believe that ten live bacteria of *E. coli* in milks, juice, burgers were enough to cause poisoning disease. While 5 Canadian people from Wellington city were dead because (1000 / 5000) drank contaminated water with bacteria (4). Some multiple-antibiotic-resistant nonpathogenic like *Escherichia coli* strains was detected in human, animal, and food origins was isolated in water and food (5). Others bacteria like *Streptococci*, which causes high fever and lungs inflammations also identified in contaminated

food (6). Other researches mentioned that some *Bacillus* species such as *Bacillus cereus*, that produce internal poisons, could cause food poisoning with vomiting and abdominal cramps during incubation period 1-6 hours and may cause death (7,8). These type of bacteria has similar symptoms of *Staphylococcus aureus*, which also isolated from milk except their difference in incubation period 8-16 hours (9). Researches described the most common off food poisoning is responsible for *Bacillus cereus*, *Staphylococcus aureus* and *E. coli* (10, 11, 12).

Cake and crisps also have a wide space in microbial contamination review, bakery of these things by machines process give a chance to grow some type of bacteria like *Staphylococcus aureus* which detected in pie and cake (13, 14). Potato products like crisps can cause many diseases related to its bacterial contamination (15). Microflora, which inoculated from soil to the potatoes and may not remove by process also considered as a source of diseases (16). Potatoes products led to increase number of gastroenteritis in United State (17).

Materials and Method

260 different samples of crisps and cakes were

collected from supermarkets, represented by 130 specimens of 26 types of crisps and the same for cakes, 5 repeated for each sample. Swap media transferred the swap from each specimen to Nutrient and Brain Heart Infusion Agar for culturing at 37°C for 18-24 hours. The plates were divided for 4 division to insure the repeated

swaps leaving one pure as control negative. Pure bacterial geniuses isolated from pure growth culture and maintain in slant at 4°C. Microbes diagnosed by microscope after Gram staining, biochemical test were done for final diagnosis as explained by diagnostic of bacteria review (18).

- Statistical Analysis were made according to ⁽¹⁹⁾ as following:-

a- **Frequency (F)**:- for frequency percent of microbes as explained in the following equation

$$F\% = \frac{\text{NO. of isolates of one microbial 's kind}}{\text{total NO. of isolates for all microbial 's kinds}} * \%100$$

b- **Occurrence (O)** :- to estimate the occurrence of microbes in each snacks samples as following :-

$$O\% = (r / N) * \%100$$

r= times NO. of microbes appearing N= total NO. of samples

c- **Distribution Intensity (DI)** :- how the microbes distributed between snacks samples by :-

$$DI = \text{Occurrence} * \sqrt{\text{Frequency}}$$

d- **Similarity** :- Total Similarity Index (TSI) were used to estimate any similarity between microbes community in both crisps and cakes.

$$TSI = a / N * \%100$$

a = NO. of the same microbes appeared in both food samples N= total NO. of isolates

e - **Jaccard 's Similarity Index (JSI)** :- This index explained how many microbes appeared in crisps only , cakes only , in both .

$$JSI = a / (a + b + c) * 100$$

a = kinds of microbes occurred in both snacks . b = kinds of microbes in crisps only .

c = kinds of microbes in cakes only .

f - **Sorenson 's Index (SI)** :- This determine the species correlation which isolates from studied samples .

$$SI = 2W / (a + b)$$

W = shared isolates in both snacks a = isolates in crisps only b = isolates in cakes only

Results

Tables 1 and 2 explained 26 different types of crisps & cakes respectively, with their various origins, flavours, kinds of microbes isolated from them. Table 3 showed 8 of different genus of bacteria distributed in 16 crisps samples and 16 cakes samples too. Six kinds of microbes in crisps, which more than in cake which was 5 only. Twenty isolates from 6 genus, arranged from the highest: Bacillus, Klebsiella, Candida, E. coli, Diplococcus and others. In addition, 20 isolates were recorded from cakes, 5 different genus appeared Bacillus was the highest followed by E. coli,

Staphylococcus, Streptococcus. Some bacteria appeared in crisps sample while disappeared in cakes. The most frequency, density and distributed genus was Bacillus 126.5 then Klebsiella 62.5 in crisps samples, their frequency 40%, 25% respectively with occurrence about 20%, 12.5% respectively. While in cakes E. coli 103.5 then Bacillus 82 were the most densities with frequency 35%, 30% respectively and their occurrence was 17.5%, 15% as explained in table 4. The total similarity index, which referred to the distribution of microbes in one or both types of snacks figure 1. Some types of crisps or cakes had more than one kinds of microbes.

Table (1) explains crisps types, their origin, flavours and microbial isolation.

Microbial isolates	flavours	origins	Crisps types
E. coli	Ketchup	Iran	Cheetooz
-ve	Vegetable	Syria	Yum Yum
-ve	Cheese	Syria	cheetooz
Candida	Lemon	Syria	Mr. Jebry
Bacillus	Chicken	Syria	Chico Abdo
Klebsiella, others	Cheese	Syria	Asmer star
Klebsiella, Candida	Peanut	Jordan	Pufak
-ve	Cheese	Jordan	Tic Tac Toe
Klebsiella	Tomato	Jordan	Baba Al- battel
Candida	Ketchup	Jordan	Yosffee
Bacillus	Pizza	Jordan	Snacks
E. coli	Salt & Vinegar	Jordan	Tickon
Klebsiella, Bacillus	Cheese	Iraq	My chips
-ve	Paprika	Iraq	Meno
-ve	Ketchup	Iraq	My school
-ve	Tomato	Iraq	Keramish baba kawi
Diplococcus, Candida	Chicken	Iraq	Shirine
Bacillus	Kebab	Iraq	Dalia
Bacillus	Cheese	Iraq	Dnia farfasha
Bacillus	Paprika	Kuwait	Crinkle
-ve	Pepper	Kuwait	Fico fesh
Klebsiella	Spicy	Kuwait	Nice
Bacillus	Natural	Saudi Arabia	Prinkles
-ve	Salt & Vinegar	Saudi Arabia	Fantzi
-ve	Salt & Vinegar	Saudi Arabia	Dream
-ve	Salt & Vinegar	Saudi Arabia	Sinono

Table (2) shows cake samples , origins , flavours , microbes isolates

Microbial isolates	flavours	origin	Cake samples
-ve	lemon	Iran	Chee.fon
Staph.	orange	Iran	Chee.fon
-ve	apple	Iran	Chee.fon
Staph.	orange	Iran	Chocó cake
-ve	cacao	Iran	Chocó cake
E. coli	cacao	Iran	Nobi
Bacillus	lemon	Iran	Yes
E. coli , Streptococcus	lemon	Iran	Teacken
Streptococcus	strawberry	Iran	Teacken
Staph	cherry	Iran	Nadi
Bacillus	banana	Iran	Razawi
Bacillus	strawberry	Iran	Topi cake
Staph , Bacillus	strawberry	Sudia Arabia	Croissant 7 days
Staph	natural	Sudia Arabia	Pain cup cake
E. coli , Streptococcus , others	cacao	Sudia Arabia	Super rou
-ve	cacao	Turkey	Luppo
-ve	strawberry	Turkey	Tat kek
-ve	cacao	Turkey	Tiny
-ve	apricot	Turkey	Kat kat tat
-ve	cream	Turkey	Pain Doro
-ve	honey	Turkey	Luppo
-ve	apricot	Turkey	Pain Doro
-ve	cacao	Greek	Cake bar 7 days
Bacillus , E. coli	cacao	Jordan	Jordina mini
-ve	Natural	Iraq	Afran Al- araies
E. coli , Bacillus	cardamom	Iraq	Al-Faisal

Table (3) numbers of microbial isolates in crisps & cakes

Total NO. of isolates	NO. of isolates in cakes	NO. of isolates in crisps	Microbial isolates
14	6	8	Bacillus sp.
9	7	2	E. coli
5	0	5	Klebsiella
4	4	0	Staphylococcus
3	0	3	Candida
2	2	0	Streptococcus
1	0	1	Diplococcus
2	1	1	others
40	20	20	TOTAL

Table (4) mentioned the occurrence , frequency, Distribution intensity for different isolated microbes

Distribution Intensity	Frequency %	Occurrence %	Isolated microbes			
cakes	crisps	cakes	crisps	cakes	crisps	
82	126.5	30	40	15	20	Bacillus sp.
103.5	15.8	35	10	17.5	5	E. coli
-	62.5	-	25	-	12.5	Klebsiella
44.7	-	20	-	10	-	Staphylococcus
-	29	-	15	-	7.5	Candida
15.8	-	10	-	5	-	Streptococcus
-	5.6	-	5	-	2.5	Diplococcus
5.6	5.6	5	5	2.5	2.5	others

Figures (1) explained similarity between isolated microbes in both snacks.

3	37.5	A	TSI
37.5	1.2	JSI	SI

A= sharing microbes in both snacks.

TSI = Total similarity index. JSI = Jaccard's similarity index. SI= Sorenson index.

Discussion

The study performed 260 randomly samples of 26 types for crisps and the same numbers for cakes. Pure culture were made from the samples by using references techniques (18). Eight microbial isolates appeared and arranged in table 1,2.The most dominance and frequency microbes as seen in table 1, were Bacillus and Klebsiella in crisps samples. As shown in table 2, E. coli and Bacillus were the most dominance in cake. These results agreed with (8,12) who mentioned “Bacillus can found in different types of food, it was the reasons of many diseases like food poisoning “.

16 crisps species were contaminated, some had more than one microbes which named (My chips; Cheetooz;

Asmer star; shirine and Bufak) recorded 19 % where as 38.5% of microbial isolates without contamination. Also 16 species of cakes were polluted with 5 different genus of bacteria as shown in table 3, 19% appeared in the same cake's samples named (Jordina mini; Croissant 7 days; super roll; Teaken and Al- Faisal) andabout 42% of cake's types had no contamination (Table 2)

Salt & vinegar crisps from Saudi Arabia were less contaminated as shown in table 1 and this may due tohigh salt & vinegar contents. Turkish cake were no contaminated as shown in table 2, perhaps for their food factories' hygiene regime.

The main causative agent of Bacillus distributionmay be due to: firstly, their production of toxins, enzymes, antimicrobialresistanceas Cerein, Zwittermicin (21). Secondly, it tolerates for high and low temperature degrees, tolerate for many antimicrobial agents (20). Finally, some researches mentioned that the virulence factors of Bacillus which form of biofilm and swarming distribution and its ability to survive in hard environment because of its spore- formation (20,22). Many researches explained dirty, unclean rule materials bad hygiene , conservatives and bad stored of food products may be cause their contaminations (14,16,17,23).

To conclude, if there is no real good attention of food hygiene , careless of food storage , conservatives,

transporting and their expire date ; there are a big probability for providing a good environment for microbes' growth and enhance their pathogenic activity

Conclusions

The study insured microbial contamination in the most studied samples of fast meal deserts crisps and cakes ,16 of 26 were contaminated for both different snacks samples .

Bacillus sp. & Klebsiella were the highest in their occurrence and frequency in crisps samples while E. coli & Bacillus sptook the first space in cakes.

Similar microbes appeared in both deserts 37.5 %.

Turkish cakes was more safety.

Salt & vinegar flavor recoded no contamination too.

Recommendations: Continues monthly examination of different food factories,stores, supermarkets to insure food hygiene and ready to use.

Export the highly healthy hygiene food products with safety conservatives.

People awareness about bad effect of damaged, can insured by holding conferences, TV. Any social media.

Apply a punishment for any responsible factories if there were any default in their food products.

Conflict of Interest : None

Source of Funding : Self

Ethical Clearance: This research was done in Basrah Technical Institute, Southern Technical University, In Barah province. The research performed samples of cake and crisps from the domestic markets. Techniques that used to isolate bacteria was in the laboratory and just normal laboratory media were used. No animal or human samples were used in this research.

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Assessment of Student's Knowledge and Beliefs about Patients' Rights

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Abstract

A descriptive study about patient rights in hospitals was employed on probability sample of 120 students in Medical Technical Institute /Al mansour (MTI) to assess students' knowledge and beliefs about patients' rights. A questionnaire was constructed for purposes of study through experience of researcher and review of literature. Collection data and analysis of results was done by frequencies, percentages and mean of scores. Results of the study indicated that there was highest percentages was reported in many of items related to rights of clinical health care as (interest and dignity treated 91.7%), psychosocial care as (family visits76.7%),general rights as (beliefs and religious in hospital' 66.6%), and highest percentages recorded by sites of internet and social media at 40.16% as a source of information. Most of the correct answers have been adopted by the sample spontaneously through non-formal information by Internet, then there is not scientific courses or lectures based on scientific rules. The study concluded that there was a clear discrepancy in level of students' knowledge and beliefs. The researcher recommended that there is need to hold awareness programs or more studies on students, patients and nurses for standing of what are knowing of patients rights and their responsibilities.

Key Words: *Student's knowledge and beliefs, Patient's Rights.*

Introduction

Human rights in patient care refers to application of patient's rights. It provides of principles of alternative to the growing discourse of patient rights^[1]. It was different in many of countries and depend on prevailing cultural, social norms and values. There are list of models of the patient and health staff like physician relationship which can represent the citizen and state of relationships have been improved today ^[2].

It may be include information about care of patient, decision making, medication nutrition and receiving truly informed consent, also rights to know name of caregivers of patient. or anything goes wrong with care, like receive false drug or some errors related to medical diagnosis or nursing care , any patient have right to be told ^[3, 4]. In addition; access to basic ranges of hospital care on principles of the clinical needs information about health and any treatment that may be required. Personal confidentiality, privacy, dignity and respect by medical and health staff. Also, give feedback, make complaints and have access to advocacy ^[5-8]

A patient should only consent to medical treatment if there is sufficient information about diagnosis and treatment options available in terms the patients can understand. before the physician can begin any course of treatment, the physician must make the patient aware plan of care , program of therapy and medical procedures, make an informed decision about case prognoses. When patients has been sufficiently informed about their treatment that provided, they has right to accept or refuse treatment ^[9, 10].

The patients have all rights to be fully informed about anything related to health status, recommended course of treatment, alternatives of care in hospital or home, benefits and risks of surgical procedures, and medical or nursing care, that involving plans of care and treatment. They may be ask any questions towards their condition, any participation in research, or entitled to a full explanation of study, including the risks, complications of treatment , benefits and alternatives of participation and their answers will honestly and very clearly. Also

all patients have right to refuse or participate in any research without any refusal affecting the care which provided and have a right to copy of medical record or sheets after discharge from clinic or hospital ^[11, 12].

Material and Method

A descriptive -study which used an assessment tool . The present study was conducted on students of MTI in Baghdad City. A Random selection of (120) students who studying in sex scientific departments. The data was collected through the interview of researcher with the sample by using a questionnaire format which constructed by the researcher. The period of the study from 1 /6 /2016 to 20 /1 /2017 in Baghdad City.

The questionnaire was consisted of three parts:

First part included demographic data of sample (3 items).

Second part included items of patient's rights:

*rights of clinical care (9 items).

*rights of Psychosocial care (9 items).

*general patients rights (9 items).

Third part concerned to sources of information (4 items).

Statistical analysis: Frequencies, percentages and mean of scores.

Finding and Discussion :

Table1: Distribution of personal- demographic

data of the sample

Variables	Frequencies (F) 120	Percentage (%) 100	
Gender	Male Female	71 49	59,2 40,8
Age (years)	19-21 21-23	67 53	55,8 44,2
Sex Scientific Departments in MTI Participation between (12,5 % to 18%)	Curriculum including patient rights	Yes No ----- 120	100

This table indicated that more than half of sample were Males (59,2%) most of them were age group of 19 to 21 years (55,8 %), and no curriculum including patient rights.

Table 2 Sample based distribution about their knowledge and beliefs towards patient rights (Clinical care).

1	Do you agree with me :	Total / 120 Responses						
		Agreement 3	Uncertain 2	Disagreement 1	MS			
		F	%	F	%	F	%	
1-1	Alternate confidence between offered care and patient	87	72,7	27	22,3	6	5	2.66
1-2	Child pat. has special treatment with confidence stubborn treated.	81	67,5	30	25	9	7,5	2.6
1-3	Feeling with mercy and sympathy includes patient and their family.	58	48,33	44	36,66	18	15	2.33

Cont... Table 2 Sample based distribution about their knowledge and beliefs towards patient rights (Clinical care).

1-4	Each case has specific approach in care plan and treatment.	85	70,8	27	22,5	8	6,7	2.64
1-5	Medicines and treatment dismissed to decision of doctor and pharmacy.	89	74,2	25	20,8	6	5	2.69
1-6	Satisfactory elucidation the case and the final diagnosis for the patient.	85	70,8	24	20	11	9,2	2.61
1-7	All patients the stubborn care and the interest and the dignity treated.	110	91,7	8	6,6	2	1,7	2.9
1-8	Presentation of the dignity is truth in the fresh sight about type , age and the case.	105	87,5	10	8,3	5	4,2	2.83
1-9	Presentation the care healthy for the patient covered legally.	84	70	26	21,7	10	8,3	2.61

This table indicated that there was moderate to high mean of scores in all statements of patient rights related to clinical care.

Table 3 : Sample based distribution about their knowledge and beliefs towards patient rights (Psycho-Social care).

1	Do you agree with me :	Total / 120 Responses						
		Agreement 3	Uncertain 2	Disagree- ment 1	MS			
		F	%	F	%	F	%	
1-1	mental saving of environment and supports for sick stubborn treated	73	60,8	43	35,9	4	3,3	2.57
1-2	respect of the believed and the values social for the patient and the family	87	72,7	22	18,1	11	9,2	2.63
1-3	Preparing required of wife sick about the examination medical	76	63,4	32	26,6	12	10	2.53
1-4	Right of patient to family visits according to instructions	92	76,7	23	19,1	5	4,2	2.72
1-5	From truth the needy patient the request in compensation when his treatment deteriorate.	78	65	30	25	12	10	2.53
1-6	The intervention does not be possible in the special patient outside frame treated	82	68,3	30	25	8	6,6	2.61
1-7	feeling of mercy and the sympathy includes the patient and his family	79	65,8	29	24,2	12	10	2.54
1-8	Consultation be necessary sick before disclosure his one of secrets for decaying	88	73,3	21	17,5	11	9,2	2.64
1-9	Secrets kept sick to in the cases which the law conflicts	83	29,2	28	32,3	9	7,5	2.1

This table indicated that there was moderate to high mean of scores in all statements of patients rights related to psycho- social care.

Table 4 : Sample based distribution about their knowledge and beliefs towards general patient rights.

1	Do you agree with me :	Total / 120 Responses						
		Agreement 3	Uncertain 2	Disagr- eement 1	MS			
		F	%	F	%	F	%	
1-1	Satisfaction of pat. do not justify doctor in work of non criminal	74	61,7	33	27,5	13	10,8	2.50
1-2	Experiments on pat. considered criminal works without desire of pat.	74	61,7	32	26,7	14	11,6	2.5
1-3	Pat. have right to oriented of instructions of hospitals	75	62,5	32	26,6	13	10,8	2.51
1-4	Pat. have rights to practices of his beliefs and religious in the hospital'	80	66,6	32	26,7	8	6,7	2.6
1-5	Patient rights is agreed upon on her about in all organizations	72	60	37	30,8	11	9,2	2.50
1-6	Pat. have to ask any questions related to his condition.	67	55,8	40	33,4	13	10.8	2.45
1-7	From right of the doctor or family sick end of life hopeless patient from his live.	75	26,5	37	30,8	8	6,7	2.55
1-8	From pat. rights sick refusal treated in any organization healthy	70	58,4	36	30	'14	11,6	2.46
1-9	From pat. rights authorization of lawyer or bringing man of debt	74	61,7	41	34,1	5	4,2	2.57

This table indicated that there was moderate to high mean of scores in all statements of patients rights related to psycho- social care aspect to general patient rights.

Table 5: Sample based distribution about level's awareness related to knowledge and beliefs of students towards patient rights.

%	Total	Level's Awareness	Items			
		High	Moderate	Low		
100	120	84	28	8	Items related to clinical rights (Table 1 = 9 items)	1
100	120	75	35	10	Items related to psycho-social rights (Table 2 = 9 items)	2
100	120	73	32	15	Items related to general rights (Table 3 = 9 items)	3

This table indicated that high scores at level of (85), moderate scores at level of (35) and low scores at level of 15.

Discussion

According to the demographic characteristics (Table 1). Results out indicated that more than half of sample were males (59,2%) were age group 19 to 21 years (55,8 %). Researcher believed this fact is more common in our colleges and institutes (males are more cooperation than females), especially when the study on students in first class. All students were distribution according to their sex scientific departments at level between (12,5 % to 18%) as result of fulfilled of purposes of the study. So, there is no curriculum related to patients rights when the researcher asks sample about such issues (Table 1). Such results supported by the same result but on nurses' Knowledge which indicated that there is no relationship between gender, age, degree and nurses' knowledge about rights of patient. It also, never passed any course^[13]. But other study reported that high percentage of students reported that only one course within their curriculum was patient's rights-course related.^[14]

Regarding to results in tables of 2, 3 and 4 there was moderate to high mean of scores in most of statements of patients rights related to clinical care, psycho-social and general rights. These results agreed with study survey which stated that high percentage between medium and high awareness but there is not significant association between knowledge and their demographic variables [15]. Another finding of study indicated that agreement too of total knowledge of students from patients' bill of rights was medium. Therefore, greater focuses should be placed on inclusion and support of patients' rights in course and curriculum planning for paramedical students^[16]. From other hand, supported by other study that reported as (Results in general, about 65% of respondents had good level of knowledge of sample, while 35% of them had bad knowledge about variables of patient rights. More than 50% of the students have good knowledge among patient rights; so results related to knowledge score is different among the senior and junior of sample)^[14].

Results in table 5 related to awareness' levels of students about patient rights concerning of clinical care indicated that high scores at level of (84), psycho-social, and moderate scores at level of (35) related to and low

scores at level of⁽¹⁵⁾ related to general rights. Such results out described good while the sample never taking course of patient rights in their curriculum. The researcher believes that most of the society has a general culture of human rights, including the patient, through the general culture. Therefore, the results were good with regard to awareness at these levels, which was noted by the study. The researcher believes that most of the society has a general culture of human rights, through the general culture and therefore the results were good with regard to awareness at these levels, which were noted by current study mechanism, This view agreed with the trends that stated like the right to health care aspects, as well as human rights related to the rights to life and freedom, to housing and work, to information and education, to human dignity and privacy and others^[17].

Regarding to source of information related to students' awareness about patient rights was Net-Social Media in percentage of 40,16 %. These results supported by study on attitudes of nurses regarding to patient rights revealed that majority of sample had received education (information) about these rights, so high percentage became aware of it through the external of school setting^[18]. For this reason one of recommendations of studies which reviewed 112 articles published for more than 20 years (other studies must be examine and assess information needs of cancers patients and its sources throughout their cancer journey)^[19]. Website of social media should be driving the direction and regularly updated with the time in order to benefit society more because these tools are considered the best tool to educate the community^[20].

Conclusion

Through the questionnaire and personal interview technique on randomized sample of 120 students of MTI in Baghdad City to identifying their knowledge and beliefs towards patient rights in hospitals. Results out of the study indicat:between moderate -high level) most of their information of students depending on Net - Social Media in most their responses.

Conflict of Interest: Non

Source of Finding- Self or other source : Self

Ethical Clearance: At the time of writing the research and personal interview with the samples was addressed to Medical Technical Institute/AI- Mansour

that need to conduct as the research registered within the annual scientific plan of the Institute and the official approvals were taken and meet with samples in this study.

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A Comparative Study between Traditional and Innovative Medical School Students Perceptions and attitude Regarding the Effectiveness of Patient Safety in Medical Education

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Abstract

Patient safety is a new discipline in a health care that determine the medical errors which leads to adverse events and it is a fundamental principle of health care. The simplest definition of patient safety is the prevention of errors and adverse effects to patients associated with health care . The study aimed is to compare between traditional and innovative medical school students perceptions and attitude regarding the effectiveness of patient safety in medical education

Introduction: Health care services have improved considerably in the light of medical advances; however, these advancements were considered as major threats to health care industry [1]. For example, in 1999 the US Institute of Medicine (IOM) estimated 44000–99000 deaths every year as a result of preventable medical errors [2]. Other studies have indicated adverse events as an international concer

Method : A cross- sectional study was carried out in both medical schools (Mosel & Tikrit) after receiving administrative agreement and a verbal consent was taken from each participant before establishing the study.

Results : Medical students from both collages and two stages were selected randomly (Third & fourth) and (180) students were participated in the study . A special questionnaire form has been designed by the investigator included five main dimensions prepared for that purpose.

The results show that medical students from third and fourth stage from traditional school go with the needed for adequate reliable reporting system for patient safety registration (47.7% and 47.3%)

Fourth stage from traditional and innovative school agree with the effective strategy to deal with the medical errors carefully as an preventive measure (60.0%, 85.7%) respectively .

Conclusions: Engagement of formal curriculum on patient safety in medical education is necessary .
Recommendations : Better policies investment for patient safety improvement with introduction of large scale studies to assess the nursing care impact on patient safety .

Key words : *Patient safety, Medical students , Perceptions , Education*

Introduction

Patient safety is the absence of preventable harm to the patient during the process of health care and decrease the risk of unnecessary risk that is related to health care to an acceptable minimum which can be defined as the collective notions of given current knowledge, resources available and the context in which care was delivered weighed against the risk of non-treatment.

..(1) . It was not known of adverse events in patient until 1990 when large number of countries mentioned that there was a medical errors affecting patients health (2) . World Health Organization (WHO) for this purpose indicated that patient safety is very important endemic concern . (1)

In deed there is a quite transdisciplinary body of both research literature and theoretical aspect that

informs the patient safety as a distinct health care science (3). The most common definition of medical error is a preventable effect of health care whether it is harmful to the patient or not.(3,4).

There are many causes of medical errors related to the followings :

A- Factors related to person because of different variation in training and experience of health providers in addition to the effect of increasing working time (5, 6)

B- Factors related to recent advances in medical field technology and prolong staying in the hospital (7, 8, 9)

There are a quite differences between patient safety and quality care because quality care concentrated mainly on better doing things with efficient and effective health care system in exact time while patient safety is the reliance on doing things without patient harm and avoiding adverse events with less and little mistakes(10)

Others defined quality care that it is the optimal balance between the realized main component (possibilities) and the frame work of standards (norms and values) (11)

The Institute of Medicine (IOM) mentioned the main component of quality care which are :

(effective safe , patient centered and equitable) (12),which should be distinguished from patient safety that meaning preventing any injury or harm to the patient(13)

The American Academy of nursing Expert panel has been worked on quality health care which mainly focused on the major positive indicators and guidelines for maintaining this care and these indicators including :

- 1- Appropriate self- care achievement .
- 2- Promotion of health care behavior
- 3- Enhancement of the quality health of life

4- The well being perception and symptoms treatment properly (14)

The study aimed to compare between traditional

and innovative medical school students perceptions and attitude regarding the effectiveness of patient safety in medical education .

Subjects and Method

1- A administrative agreement

Official permission was taken from Mosel & Tikrit medical college before starting the study and a verbal consent was taken from each student .

2- study design and setting :-

The study was a cross- sectional which was carried in Mosel & Tikrit medical college .

3- Study sample and sampling method :-

One hundred eighty (180) medical students from both stages (3th and 4th) were selected randomly during their study after taken a verbal consent from them before participating in the study with full complete description of the study aim was done by the investigator .

4- Study period :

The data was collected by interviewing with the study students during the period from 1st February / 2014 till the end of May / 2014 .

5- Data collection tool :-

A special questionnaire form has been prepared by the investigator utilizing available related literature which include the following main items :

Part-1- Demographic characteristics including (sex, age, stage).

Part-2- Students perceptions assessment about patient safety in medical education .

Part-3- Students attitude about the main causes and the importance of patient safety .

Part-4- Students perceptions regarding the preventable measures of medical errors .

Part-5-Student perceptions about the engagement of formal curriculum on patient safety in medical education .

6- Reliability of the questionnaire form :

The questionnaire was presented to (4) experts in the medical education , they were (2) community physicians and (2) statistical experts .

The reliability of the questionnaire was 70 % .

7- Statistical analysis of data :-

The data was statistically analyzed by :

a- Descriptive statistics for questions with yes and no answer by using (number, percent).

b-The statistical test Chi—square was used and p value was considered significant at the level of ≤ 0.05 .

c-The items of students attitude assessment were rated according to type of likert scale as (yes- uncertain- no) and scored as :

3 for yes answer

2 for uncertain answer

1 for no answer

Cut off point $(3+2+1) / 3 = 2.0$, so the results calculated by using the following formula(15) :

No. of students said yes $\times 3$ + No. of students said uncertain $\times 2$ + No. of students said no $\times 1$

Sample size (180)

Finding

Table (1) : Socio demographic characteristics of the study medical students

Socio demographic parameter		Traditional school (Mosel) N=120	Innovative school (Tikrit) N =60	Total N= 180	
		No. %	No.	No.	%
Sex	Male	49	17	66	36.7
	female	71	43	114	63.3
Age group (in years)	22-24	109	53	162	70.0
	25-27	8	5	13	7.2
	>27	3	2	5	2.8
Stage	Third	65	39	104	57.8
	Fourth	55	21	76	42.2

Table 1 shows that most of the study students are female(114- 63.3%), aged between 22-24 years of age (162- 70.0%) .

Table 2: Distribution of study students according to their perceptions regarding patient safety in medical education

Students perceptions regarding patient safety	Study students N= 180								P* Value
	Traditional school (Mosel)N=120				Innovative school (Tikrit)N=60				
	Third stage		Fourth stage		Third		Fourth		
	No.	%	No.	%	No.	%	No	%	
Patient safety should be well informed	11	16.9	7	12.7	9	23.2	2	9.6	0.462
The needed for a adequate reliable reporting system for patient safety registration	31	47.7	26	47.3	5	12.5	4	19.0	0.000
A good successful estimation of patient hospitalization with adverse events	15	23.1	12	21.8	6	15.3	4	19.0	0.807
Medical errors must be noted by specialized body	8	12.3	10	18.2	19	48.7	11	52.4	0.000
Total	65	100.0	55	100.0	39	100.0	21	100.0	

* χ^2 – test was used

Table 2 indicated that both medical students from third and fourth stage from traditional school go with the needed for adequate reliable reporting system for patient safety registration (31-47.7% and 26-47.3%) respectively with a p- value = 0.000.

On the other hand students from innovative school (third & fourth stages) go with that the medical errors must be noted by specialized body (19-48.7%, 11-52.4%) with a p value = 0.000.

The national quality form (NQF) mentioned in their work to bring the clear description of patient safety and its standardization taxonomy ⁽¹⁶⁾ . It defines harm as the failure of health care process and the impact of harm either temporarily or permanently , physiologically or psychologically or affecting body structure and function.

Gilberto etal ⁽¹⁷⁾ study among 130 fourth year medical student in the University of Hong Kong, Li Ka Shing Faculty of Medicine, Hong Kong / during July /2009 to assess their perceptions and knowledge about patient safety . They found that most of students go with that the medical errors were inevitable but 25% of them said that efficient physician cannot do any errors and majority of them suggested a necessary strategy for reporting the medical errors to prevent its occurrence . They concluded in their survey that medical students show their agreements with the multidisciplinary approach for medical errors management and 10% of them did not agree with establishment of notifying reporting center .

Table (3) :Frequency distribution of study medical students according to their attitudes towards the main causes and importance of patient safety in medical education

Students attitude towards patient safety	Number of study students (=180) Traditional & Innovative			Mean of score	Grand mean (CM)/ assessment value
	yes	uncertain	No		
<u>Causes of medical errors :</u>					
a- Training gap in health care provider	112	17	51	2.3	GM= 2.1 Above cut- off point
b- Technology advances	89	43	48	2.2	
c- Work time pressure	95	18	67	2.1	
d- Obscure authority line of physician and other health staff	47	59	74	1.8	
<u>Importance of patient safety in medical education :</u>					
a-It is the cornerstone of high quality care	87	35	58	2.1	GM=1.8 Below cut-off point
b-Emphasis on error prevention and learning from these errors	28	41	111	1.5	

Table 3 show that medical students attitude regarding the causes of medical errors was above cut-off point(2.1) and low regarding the importance of it in medical education (1.8)

Nabilou etal/2012 ⁽¹⁸⁾ conducted a similar study among medical students in Iran / West Azerbaijan province/ in teaching hospitals affiliated with Urmia University of Medical Sciences (UUMS)to assess their perceptions about the patient safety .

They found that 80% of medical students agree that providing healthcare is not the possible preventable measure and 60% of them said that medical error are unavoidable . On the other hand 64% of students agree that the main causes of errors are because of the

physician who cannot do any thing to avoid occurrence while another 64% of them are disagree that these errors are not caused by health care providers .

Wakefield etal / ⁽¹⁹⁾ mentioned in their study about the role of nursing and medical curriculum in addressing the patient safety in the United Kingdom (Department of Health, 2000/ An organization with a memory) to examine to what extent patient safety is addressed within medical and nursing curricula in order to managing these errors in effective manner and they recommended that future physicians should work and learn the impact human limitation on clinical field through the improvement of health care and related its burden on the patient .

Table 4:- Medical students distribution according to their perceptions about the preventable measures of medical errors

Students perceptions regarding the preventable measures of medical errors	Study students N= 180				P* value
	Traditional N= 120		Innovative N=60		
	Third	Fourth	Third	Fourth	
Address the patient error routinely whether harm or not	15 (23.1%)	10 (18.2%)	15 (38.5%)	1 (4.8%)	0.077
Effective strategy to deal with these errors carefully	37 (56.9%)	33 (60.0%)	5 (12.8%)	18 (85.7%)	0.000
Medical errors treated constructively	13 (20.0%)	12 (21.8%)	19 (48.7%)	2 (9.5%)	0.007
Total	65	55	39	21	

* χ^2 – test was used

Table 4 presents that both fourth stage from traditional and innovative school agree with the effective strategy to deal with the medical errors carefully as an preventive measure (33- 60.0%, 18-85.7%) respectively in comparison to (19- 48.7%) from third innovative school agree with the treatment of medical errors constructively with a P value =0.007.

A similar study was done by Hamdi et al / 2010⁽²⁰⁾ in Saudi Arabia to assess the knowledge and attitude towards patient safety among group of undergraduate medical students by selecting two medical schools, one follow the traditional learning through lecture – based and the other one applied innovative method of teaching. They found that 52.7% of the participants students

having a good knowledge about the patient safety and 60.7% of them agree with importance of it in medical education and majority of them go with the need for supporting peers who make the unintentional medical errors and it should not be blame them for these mistakes. They mentioned that less than half of students (44.7%) agree with the role of patient as one of effective method and strategy in preventing medical errors.

On the other hand Seiden et al /⁽²¹⁾ presents in their study about the role of medical students in preventing patient harm that majority of students were a good participants in maintaining patient safety when given their essential adequate training and opportunities.

Table 5:- Frequency distribution of study students according to their perceptions regarding the engagement of formal patient safety curriculum in medical education

Students perceptions regarding the engagement of formal patient safety curriculum in medical education	Study students N= 180				P* value
	Traditional N= 120		Innovative N=60		
	Third	Fourth	Third	Fourth	
Agree	41 (63.1%)	26 (47.3%)	23 (58.9%)	8 (38.1%)	0.235
Disagree	24 (36.9%)	29 (52.7%)	16 (41.1%)	3 (61.9%)	
Total	65	55	39	21	

* χ^2 – test was used

Table 5 show that third stage from both traditional and innovative school agree with the engagement of formal curriculum on patient safety in medical education (41-63.1%, 23-58.9%) respectively in comparison to (29-52.7%)from traditional school (fourth stage) disagree with it with a P value =0.235.

Madigosky et al / ⁽²²⁾ recommended in their study about the changing and sustaining medical students' knowledge, skills, and attitudes that patient safety and medical fallibility that when comparing the students impact before and after introduction of information about the formal patient safety curricula . They found that there was a good improvement in their knowledge , skills and awareness .

Anne et al / ⁽²³⁾ published in their study on core competence for patient safety research during 2008 through a process involved a multistage initial frame work among 35 countries reviewing the related existing literature by conducting a consultation series and international experts to reducing the patient harm through out the world . They reported that there should be a long-term continuous efforts to maintain the practical capacity for research solving the local problems and improves the patient safety .

Conclusions

1- There is an agreement about no successful estimation of patient hospitalization .

2- There was a high level of students attitude about causes of medical errors and low regarding importance of patient safety in medical education .

3- Medical errors can be prevented by effective strategies .

4- Formal curriculum on patient safety is necessary in medical education.

Conflicts of interest : Non

Ethical clearance: a self verbal agreements were taken from the students before the study

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Clinical Study of Some Bacteria and Fungi that Cause Vaginitis in Al-Khalis City

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Abstract

The study included collecting (105) samples from women complaining of clinical symptoms of bacterial vaginitis, distributed into 80 samples from pregnant women (25) samples from women non-pregnant women, with their ages ranged between (15-50) years during the period from 2015 to 2016. After laboratory diagnosis, the results showed that the higher number and percentage of bacterial vaginitis was among pregnant women 60(72%) when compared with non-pregnant women 9(36%). Bacteriological examinations of the isolates showed that the highest rate of infections was caused by *Gardnerella vaginalis* (51.5%), followed by *Staphylococcus aureus* (22.5%), and *Klebsiella* (10%), while fungal infection rate with candida was (15%). The highest rate of vaginitis was among the age group (21-27) years (41.9%), followed by the age group (15-21) years (19.0%) due to sexual activity and high hormonal levels. The sensitivity test of the studied bacterial isolates was done against 8 antibiotics, and all bacterial isolates were shown to be resistant to Nalidixic acid and metronidazole, while most isolates were sensitive to Gentamycin and Cephalotoxin.

Key words: clinical study ;vaginitis ;*Gardnerella vaginalis* ;candida; pregnant women infection.

Introduction

Vaginitis is one of the most common sexual diseases in women during pregnancy and childbirth^[1]. Hormonal changes play a key role in changing vaginal normal flora leading to bacterial infections because the normal levels of estrogen hormone is important to maintain vaginal balance and vaginal resistance to bacterial infections as this hormone stimulates and activates the development and integration of the vaginal squamous membrane^[2]. There are many causes of vaginitis including bacterial causes which is termed as bacterial vaginitis, which reflects the imbalance state and interference between the anaerobic bacteria causing infection as well as the lack in lactobacillus, which is the vaginal normal flora^[3]. Vaginitis can be caused by fungi and yeasts, while parasites and viruses are also other causes of the disease^[4].

The inaccurate diagnosis of women with vaginitis and hasty treatment without performing the sensitivity test and all laboratory tests may lead to the emergence of bacterial strains resistant to antibiotics^[5]. The random use of antibiotics also causes production of antibiotic-

resistant bacterial strains, with usual prevalence of resistance features^[6]. The present study aim to detect vaginitis causes in pregnant women. Bacterial vaginitis is the most common cause of abnormal vaginal discharge among women at reproductive age, and is the main cause of urinary tract infection^[7]. The female reproductive system is more susceptible to infection than the male reproductive system because their urethra is short and close to the anal opening^[8]. The main source of bacterial vaginitis is the normal flora present in vagina and faces or when they colonize the vagina and urethra^{[9][24]}.

Materials and Method

Sample collection

One hundred five, vaginal swab samples were collected from pregnant and non-pregnant women, who were complaining of vaginitis symptoms, with their ages ranged between (15-50) years in Al-Khalis city–Diala governorate during the period from 2017 to 2018. The vaginal swabs were cultured on Columbia agar, Mannitol salt agar, Chocolate agar, Blood agar and Mackonkey agar, and incubated at 37C for 24-48 hours.

At the same time the samples were culture on potato Dextrose Agar to detect fungal identification after that bacterial growth was examined and the positive cultures were sub cultured. The isolates were diagnosed according to ^[10] depending on the bacteriological and biochemical results.

Antibacterial susceptibility Test

Antibiogram was carried out for all isolates (105) on muller- Hinton agar. Wich used some Antibiotic were(8)the results compared^[11].

Serological tests

From all studied women, (5) ml of blood were withdrawn and serum samples were separated by centrifugation of clotted blood for 5 minutes at 3000 RPM. Serum samples were distributed in two Eppendorf tubes and placed in deep freeze to do the following serological tests:-

VDRL (Venereal disease research laboratory) test according to following immunologic method:

50 µL of each of the patient's serum, positive and negative controls were put in Khan tubes.

20 µL of Carbon suspension reagent was added to each drop.

Tubes were shaken about (80-100) movements / minute for 8 minutes using Kahn shaker, and observed for agglutination.

Finding and Discussion

Isolation and identification: Results of the current study revealed that the highest number and percentage of bacterial vaginitis was among pregnant women 65 (81.25%) when compared with its number and percentage among non-pregnant women 16(64%) as shown in table and figure (1).

Table (1) Numbers and percentages of bacterial vaginitis in pregnant and non-pregnant women

Group	Number	Percentage
Pregnant women	65	81.25%
Non - Pregnant women	16	64%

Bacteriological examinations of the swab specimens showed that the highest rate of vaginitis-causing isolates was *Gardnerella vaginalis* 42(52.5%), followed by *Staphylococcus* 18(22.5%), followed by *Candida* isolates 12(15%), followed by *Klebsiella* 8(10%), as shown in table (2) and figure . These results largely coincide with the findings of [8]. who also isolated *Staphylococcus aureus* and *Gardnerella vaginalis* from women infected with vaginitis. Table and Figure (2).

The r results agree with ^[12] who showed that bacterial vaginitis was the highest among pregnant women in their study^[13] .indicated that the most important cause of recurrent bacterial vaginitis during women's life is the microbial imbalance between the commensal microorganisms which form the normal flora, leading to an overgrowth of a type of these normal flora versus a decrease in the growth of other types, and to a reduction in the number of bacteria that have inhibition effect on other microorganisms, as well as other causes such as random antibiotic administration, contraceptives etc. which promote bacterial infection with appearance of pathological symptoms like vaginal discharges, itching, change of PH value and release of vaginal odor^[14].

Table (2) Percentage of bacteria causing vaginitis in pregnant

Bacteria isolated	Number	Percentage
<i>Gardnerella</i>	42	52.5%
<i>staphylococcus</i>	18	22.5%
<i>Klebsiella</i>	8	10%
<i>candida fungi</i>	12	15%
Total		100%

Distribution of women according to age groups

The ages of the women who were enrolled in this study ranged between (15-50) years, distributed into six groups as seen in table and figure (3).

Table (3) shows the age groups of vaginal injuries by age groups of women infected

Age	Counting the tested specimens	Percentage
15-21	20	19.0 %
21-27	44	41.9 %
27-33	15	14.3 %
33-39	12	11.4 %
39-45	9	8.6 %
45-50	5	4.8 %
Total		100%

The highest percentage of bacterial vaginitis (41.9%) was recorded in the age group (21-27) years, followed by the age group (15-21) years (19.0%), followed by the age group (27-33) years (14.3%), followed by the age group (33-39) years (11.4%) and followed by the age group (39-45) years (8.6%), while the percentage of vaginitis incidence among patients with ages over than 45 years was the lowest (4.8%). In comparing our results with others regarding age, it is demonstrated that they were in agreement with the results of [15] . who reported a higher infection percentage (19%) in women at reproductive ages within the second age group, with a decrease in vaginitis incidence with aging within the age group (>45) years [16]. indicated that age is one of the factors responsible for the change in vaginal normal flora and causes periodic appearance of some pathogens. The cause of high vaginitis rates in the younger age groups (15-21) and (21-27) years is attributed to the fact that these ages represent early marriage ages where sexual activity increases, as well as the highest levels of reproductive hormones in these ages. While in older ages (>45 years), sexual activity is reduced and the PH value becomes lower i.e acidic [16]

The causative agent of vaginitis in different women conditions

The study was conducted on samples taken from (105) women patients with clinical symptoms of bacterial vaginitis. Study samples were distributed into (80) pregnant women and (25) non-pregnant women who were complaining of vaginitis due to overgrowth

of some pathogenic bacteria including *Gardnerella vaginalis*. Table and figure (4) indicates that the number and percentage of vaginitis caused by *Gardnerella vaginalis* among pregnant women was 35(43.3%), and among non-pregnant women was 14(13.3 %). These results were almost similar to the findings of [17]. who studied vaginitis in (140) women and found that *Gardnerella* was the causative pathogen in (39%) of them, while *Staphylococcus aureus* was the causative agent of vaginitis in 15(14.3%) of pregnant women and in 7(6.7%) of non-pregnant women, which agree with a study that revealed (14%) infections in pregnant women and rendered it to the high vaginal PH resulting in an increase in the number of bacteria [17]. In regard to vaginitis caused by *Klebsiella*, table (4) shows that 10 (9.5%) cases of pregnant women and 5(11.4%) of non-pregnant women had vaginitis due to *Klebsiella*, and these results are very low in comparison with other studies conducted on pregnant women infected with vaginitis and found that *Klebsiella pneumonia* was isolated from (51.6%) of vaginal swabs taken from women infected with vaginitis [18].

Table (4) cases of women under study with type of disease

Pregnant	preg-nant	Pregnant	Pregnant
Gardnerella	49	pregnant 35	33.3
		Not pregnant 14	13.3
Staphylococcus	22	pregnant pregnant 15	14.3
		Not pregnant 7	6.7
Klebsiella	15	pregnant 10	9.5
		Not pregnant 5	4.8
candida fungi	19	pregnant 12	11.4
		Not pregnant 7	6.7

The sensitivity test was performed to the studied (105) vaginal isolates against (7) antibiotics as shown in table (5). The results revealed that bacterial isolates of *Gardnerella vaginalis* showed the highest resistance (100%) to Nalidixic acid and metronidazole, while these isolates showed the lowest resistance (11.1%) to Gentamycin. Isolates of staphylococcus aureus

showed the highest resistance (60%) to Nalidixic acid and metronidazole, while they showed the highest sensitivity (20%) to Penicillin, Ampicillin, Gentamycin and Imepenim. In this study, klebsiella isolates showed (100%) resistance to Nalidixic acid and metronidazole antibiotics, while they demonstrated high sensitivity to other antibiotics. Results of the current study showed that all bacterial isolates have the highest resistance characteristics against Nalidixic acid and metronidazole antibiotics, and this finding is rendered to the commensal relationship between these bacteria and *Bacteroides* species in cases of undifferentiated bacterial vaginitis, which are highly sensitive to these two antibiotics to

kill the anaerobic bacteria, which allows the death of the *Gardnerella vaginalis* bacteria by other defense mechanism^{[19][20]}. as well as the random and unlimited use of antibiotics. The results of our study showed that most bacterial isolates were sensitive to Gentamycin and Cephalotoxin, and this may be attributed to absence of plasmids in these isolates, and these plasmids are known to contribute markedly in increasing the bacterial resistance to antibiotics^{[21][22][23]}. Results of this study were consistent with^{[24][25][26]} who found that their isolates showed (10-18%) sensitivity to Cephalotoxin, Impenin and gentamycin^{[27][28][29][30]}.

Table (5). Resistance of bacteria against Antibiotics

Antibiotic Bacteria	Metronidazole 500 µ/ml		Nalidixic acid 500 µ/ml		Gentamycin 10 µg/ml		Ampicillin 500 µg/ml		Penicillin 500 µg/ml		Imipenem 10 µg/ml		Cephalothin 500 µg/ml	
	R	S	R	S	R	S	Res	S	R	S	R	S	R	S
<i>Gardnerella vaginalis</i>	100%		100%		11.1%									
<i>Staphylococcus aureus</i>	60%		60%		20%		20%		20%		20%			
<i>Klebsiella</i>	100%		100%											

R: Resistance S: Sensitive

Conclusion

Vaginitis take place in patients of this study especially pregnant women in highest rate of infection comparison with other non-pregnant women. *Gardnerella vaginalis* was more causal agent of vaginitis comparison *Staphylococcus aureus*, *Klebsiella pneumonia* and *Candida albicans*. The drug choice that was more effect against bacteria was metronidazole.

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Ethical Clearance:- Formal administrative approval was obtained from laboratory Al-Khalis hospital.

Conflict of Interest: None

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Sero-prevalence of Helicobacter Pylori among Staff & Students in Faculty of Pharmacy

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Abstract

Background: Helicobacter pylori (H.pylori) is a kind of bacteria that inhabits in the gastrointestinal tract and has a tendency to attach the gastric lining of stomach. The infection with H.pylori is commonly harmless, but it is responsible for the majority of ulcers in the gastrointestinal gut. In addition to, it is a major pathogenic cause for gastro duodenal ulcer and gastric carcinoma. Since of fluctuating epidemiologic conditions and changing the resistance patterns with therapeutic implications, the medical controlling of H.pylori infection is still dynamic process and need periodic caring. **Objective:** to investigate the sero-prevalence of H. pylori infection among students and staff from faculty of pharmacy / Kufa University (Najaf, Iraq) as a foundation for early detection for this infection and identify the epidemic state of this infection between selective people. **Methods:** 90 serum samples collected from students and staff, then they tested for anti-human IgG using Combo one-Step Rapid Test Cassette. The two distinctive red lines showing in the control and regions of the kit after 10 minutes indicated positive reaction. **Results:** Of the 90 blood samples enrolled for this study, serological evidence for the prevalence rate of H.pylori infection was 26 (28.9%). Analysis of seroprevalence of infection revealed that the prevalence was higher in female (33.3%) than in male that appeared less percentage. In addition, the results of this study showed that the high percentage (54.5%) of H. pylori infection appeared in age more than 35 years, besides blood group A has more likely to be infected with H.pylori. **Conclusions:** The detection of Helicobacter pylori infection using blood test found that moderate to low prevalence among staff and students in faculty of pharmacy / Kufa University.

Keywords: Helicobacter pylori, seroprevalence, age, gender, blood group.

Introduction

Helicobacter pylori, a spiral flagellate bacteria, Gram-negative, living in the gastric mucus adherent to the mucosa layer. It has an influence on approximately 50-60% of the human population, with a high incidence in those living in developing areas. Several studies reported that, the acquisition of infection in an early childhood (30-50%), which reaches up to 90% through adulthood in developing countries. This might be associated to the overcrowded and economic condition (1). Moreover, H. pylori is the predominant pathogenic bacteria in the stomach, which may cause chronic disease that can lead to numerous disorders like gastritis, peptic ulcer, gastric cancer, as well as B-cell mucosa-associated lymphoid tissue (MALT) (2,3). Correspondingly, H. pylori infection enhance the immune response by increasing the inflammatory factors such as interleukin

1 (IL-1), interleukin 8 (IL-8), and tumor necrosis factor –alpha (TNF- α) that may result in metabolic changes. (4, 5)

Epidemiologic researches have shown that half of industrialized countries and nearly 90 percent of adults in these countries are seropositive for serum antibodies against H.pylori (6, 7). Moreover, serologic outcomes reported that H.pylori infection is seldom occurred before ten years, but rises to 10 percent in those between 18- 30 years and half percentage in older than age 60 years (8, 9)

Even though the exact modes by which H. pylori bacteria infects an individual remains unknown, but it has been thought that person-to-person transmission is most likely way through direct contact with stool or saliva. As well as contaminated water supplies may serve as an environmental source of the bacteria and eating

salty food that possibly will increase the persistence of *H. pylori* infection. (10)

نيرب اصل دعو مللا نا ميحرلا نمحرلا مللا مسب
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Material and Method

This study carried out in the faculty of pharmacy / Kufa University/ Iraq. The major aims in our study were to detect and find the predominance of *H. pylori* infection among staff and students. The study approved by the ethical committee of clinical laboratory sciences department, faculty of pharmacy, Kufa University. As well as from all participants.

Sample collection

The blood samples (whole blood) were collected from all participants who agreed to enroll in this study. All individuals were required to fill the questionnaires and data form to provide necessary information such as age, gender, and blood group.

Serological test

Blood samples withdrawn from each person, a sterile dispensable dropper transferred 3 drops of the blood (approximately 100 mL) each time to the wells of the test kit (*H. pylori* Antibody kit. CLIA waived. USA).

In this procedure as showed in figure (1), anti-human IgG is immobilized in the test line region. Then, specimen added to the specific well of the device of test, the reaction with *H. pylori* antigen coated particles in the test after 2-3 minutes. This mixture transfers chromatographically along the length of the test and interacts with the immobilized anti-human IgG. If the specimen has *H. pylori* antibodies, a colored line will appear after ten minutes, which is indicating a positive outcome, on contrary, the specimen does not have antibodies; a colored line will not appear

demonstrating a negative result as presented in figure (2).

Figure (1) H-Pylori detection kit procedure

Figure (2) illustrate the serological results interpretation of *H. pylori* detection

Statistical analysis

Statistical analysis of data achieved by Chi-square test using SPSS software (version 22) to eliminate the sero-prevalence rate of *H. pylori* infection between groups of this study and the significant results interpreted in p -value less than 0.05.

Findings

A total of 90 staff and students recruited in this study, the characteristics of study population was shown in Table (1), 51 males (57.8%) and 39 females (43.3%) with age varied from 17 to 45 years. The results revealed that, the *H. pylori* seropositive result for population study was (26) with seroprevalence rate as (28.9%). Among them, the seropositive infection rate among male was 25.5% compared to 33.3% among female. The statistical analysis showed there is no significant difference in infection between gender in ($p < 0.05$). Whereas, the infection rate was revealed the significant associated with age as shown in Table (2), the group aged 35-45 years had the highest rate (54.5%, 6/11), while the group aged 17-25 years had the lowest rate of infection (22.3%, 15/67). Although, there were five seropositive results aged from 25-35 years old. On the other hand, more analysis on ABO blood groups with seropositive of *H. pylori* infection confirmed that positive results rate of infection was (31.4%) in blood group A, followed by blood group O (29%), 28% in blood group B, and lowest rate in blood group AB (16.6%). In association of the subjects have blood group A with the other blood groups as presented in Table (3), the statistical differences in ($p < 0.05$) could be recognized in the seropositive of *H. pylori* infection.

Table 1: Base line characteristic of subjects infected with H. pylori.

Variable	Total number
Gender	90
Male	51 (57.8%)
Female	39 (43.3%)
Age	
17-25 yrs	67 (74.4%)
25-35 yrs	12 (13.3%)
35-45 yrs	11 (12.2%)
Blood group	
A+	35 (38.8%)
B+	25 (27.7%)
AB+	6 (6.6%)
O+	24 (26.6%)

Table 2: Number of helicobacter pylori positive according to age group

Age group	Total number
17-25 yrs	15
25-35 yrs	5
35-45 yrs	6

Table 3:Rate of helicobacter pylori positive according to ABO blood group

Blood group	Percentage
A+	31.4 %
B+	28 %
O+	29 %
AB+	16.6 %

Discussion

Helicobacter pylori infection is currently affecting more than half of world's population (11), which makes this infection resemble the major public health concern. However, a little known about its prevalence (12). The prevalence of seropositivity of H. pylori infection is variation between countries also within the same country owing to socioeconomic status that is being greater among individuals with lower economic condition. Accordingly, infection may rely on several variables like sex, age, dietary habits, socioeconomic situation, immunological and genomic factors. (13)

Currently, our study reported that the prevalent rate of positivity for H. pylori disease was (28.9%) between staff and students in College of Pharmacy/University of Kufa/Iraq, which is less than the average predominance in the world's population (50%). Conversely, this seroprevalence rate was less than that reported in another study that showed the prevalence of this illness was 55.8% among population of university students' in Iraq (14). This variation may be due to our study screened small sample population, and may be they have different lifestyle from other community. It is interesting to note that, the prevalence rate of serum antibody to H. pylori observed in current study is comparable with other study that reported by Hanafi and Mohamed. (15) The outcomes also in accordance with several studies from developed countries that have shown conclusively that pylori infection prevalence started to decline in Asia. (16)

Furthermore, the analysis revealed that the seropositive rate of infection among females were (33.3%) whereas males have lower prevalence rate (25.5%), as realized by some several studies (17). A number of studies have observed no connection between Pylori infection and gender while other have noticed a higher prevalence of H. pylori in males. Our findings appeared no substantial noteworthy difference in H. pylori frequency with gender. However, data is compatible with other studies (18) and outcomes of Tarkhashvili et al. (19) and Shokrzadeh et al. (20) that they showed female students are more disposed to H. pylori than male students, whereas, this data was in contrary to others. (21)

Based on age, the bacterial infection observed a significant relation between age and H. pylori prevalence, with the highest infection rate in the age groups 35-45

years and lowest rate was in young population aged 17-25 years, therefore, infection with *H. pylori* may increase with age. This study showed similar outcome with previous studies that reported the high prevalence rate of pylori infection mostly in the elderly age that may be due to more exposure to this infection through life and constantly accumulating risk factors (22, 23). This outcome indicates that the major public health problems of disease in the society that may be difficult to be eradicated.

Previously it has consistently demonstrated that blood group antigens are associated with progress of peptic ulcer and gastric cancer, (24,25) therefore, current study revealed that the participants with blood group A were found to be more prone to infection than individuals in other groups ($p < 0.05$). Our outcomes supported with other previous conclusions (26). On contrast, the observation in present study is not agreed with previous demonstrations (27), which reported that the O blood group individual are higher susceptibility to *H. pylori*, as a result of greater secretor status like (H-antigen) which is an important receptor that expressed in gastro-duodenal mucosal cells and helps pylori bacteria to adhere and enhance to colonize (28).

Conclusion

In conclusion, this paper has highlighted that *Helicobacter pylori* infection has moderate to low endemic prevalence that should be aware for this infection as a possible cause of illness such as peptic ulcer and gastric cancer in adults. In addition, it can be concluded that females and adolescents are more exposed to affect with *Helicobacter pylori* infection, as well as, individuals with blood group type (A) are further susceptible for this infection. These important points indicate to the public health concern and need more investigations and further study.

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Conventional and molecular diagnosis of oral candidiasis in Iraqi Patients

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Abstract

Oral candidiasis is the most common human fungal infection in babies and elderly. The infection may pass to babies during breastfeeding (milk of mother). In elderly the infection occurs among persons who wear dentures, diabetes mellitus and is a common problem among the immunocompromised. This study aimed to isolate and diagnose of the oral candidiasis using conventional and molecular methods. (50) samples have been collected from patients suffering from oral candidiasis who attended AL-Zahra Hospital in AL-Kut city, Iraq, during (August 2017 to February 2018). Samples were CHROMagar culture and API 20 Candida stripe for initial identification of *Candida* isolates. Germ tube test, chlamydospore formation DNA isolates was extracted for polymerase chain reaction (PCR).

A total of 60 isolates were obtained from oral samples. *C. albicans* accounted for (43.3%) of the strains including single and mixed infections. The other identified species were *C. krusi* (28.3%), *C. tropicalis* (11.6%). Mixed infection with two species of *Candida* was recorded in 12 % of Oral candidiasis. The most common mixed infection was the combination of *C.albicans* and *C. glabrata*. *C.albicans* was the most common cause of Oral candidiasis. The study shows that CHROM agar was a good tool for initial identification of *Candida* isolates and PCR method confirmed of *Candida* isolates.

Keywords:: oral infection, *Candida*, PCR, fungal infections, babies and elderly.

Introduction

Oral candidiasis is an opportunistic infection of the oral cavity. It is common among infants who feeding from mother (breastfeeding) suffering from Breast thrush and elderly, particularly in those who wear dentures and in many cases^[1]. It is avoidable with a good mouth care regimen^{[1],[2]}. It can also be a mark of systemic disease, such as diabetes mellitus and is a common problem among the immunocompromised^[2]. Oral candidiasis is the most common human fungal infection especially in early and later life^[3].

C. albicans is a normal commensally of the mouth and generally causes no problems in healthy people. Overgrowth of *Candida* however, can lead to local discomfort, an altered taste sensation, dysphagia from oesophageal overgrowth resulting in poor nutrition, slow recovery, and prolonged hospital stay^[4]. There are many of Risk factors for developing oral candidiasis: pathogen has peculiar properties that increase its infectivity rate in the right environment, therefore *Candida* can be

found on or in the human body with the gastrointestinal tract, the vagina, and skin being the most common sites and *C. albicans* being the commonest species isolated from these sites^[5]. The second factor is host factor that could be local or systemic. local factors include wearing dentures, impaired salivary gland function, inhaled steroids, and oral cancer. While the Systemic factors include extremes of age, smoking, diabetes mellitus, Cushing's syndrome, immunosuppressant, malignancies, nutritional deficiencies, and antibiotic^[6].

Materials and Method

Sample collection and analysis

Samples were collected from 50 patients suffering from oral candidiasis (20 men, 25 women; average age 50-65 years and 5 babies less than 1 years) who attended AL-Zahra Hospital in AL-Kut city, Iraq, during the period from August 2017 to February 2018.

Culture Media

The patient swabs were inoculated on to plates of sabouraud dextrose agar (oxoide) .^[7] The plates were incubated at 37 °C for 72 h. the yeasts on the media were examined under low power magnification for the presence of budding cells, hyphae, blastospores, and chlamydospores. Those which contained chlamydospores were considered *Candida albicans*. Carbohydrate assimilation test was done on non-albicans species, using the purified single colonies on Sabouraud agar that were streaked on Chromoagar (Biomérieux, France) and incubated for 24-48h. isolates of *Candida* were classified according to the colors on Chromoagar based on colored key designed by^{[8][9]}. API 20 *Candida* stripe was done for initial identification of *Candida* isolates. Germ tube test, chlamydospore formation were also performed to reinforce identification.

Extraction of DNA

Extraction of genomic DNA was done and all samples were tested for the presence of genomic DNA in all *Candida* isolated and other microorganisms suspected *Candida* spp. Deoxyribose nucleic acid of *Candida* spp. was extracted by picking single of colony using sterile loop and suspended into (300 µl) of lysis buffer [10 mM Tris, 1mMEDTA (pH=8), 1% SDS, 100 mM NaCl, 2% Tween 80], 300 µl phenol-chloroform (1:1); it was shaken for 5 minutes and centrifuged at (1000 rpm). Dry DNA pellet was re-suspended in 100 TE buffer and stored at -20°C until use^[10].

Polymerase chain reaction PCR

The universal primer pair sequences that were used in conventional PCR to detect the presence of the 18S rRNA gene of *Candida* spp. was taken from^[11]. And a specific primer pair sequences to detect the presence of *C. albicans* based on the sequence data for the (ITS) region, was selected and synthesized in Alpha DNA® (Canada) as shown in table No.1. DNA template of *Candida* was prepared^[12]. The primers (*Candida* spp and *C. albicans*) were diluted by adding nuclease free water according to the manufacturer instructions. The master mix contents were thawed at room temperature before use, and the PCR master mix was made on a separate biohazard safety cabinet with wearing hand gloves at all times to avoid contamination. For each reaction within each single pre-mixed PCR reaction

tube, 2µl from each forward primer and reverse primer were added. PCR mixture without DNA template (non-template negative control) were used as negative control. The tubes were placed in PCR machine and the PCR program, and the right cycling conditions pre-installed, was started. Cleaver Scientific Thermal Cycler TC32/80 was used for all PCR amplification reactions. The PCR thermocycler program used with 18S rRNA gene of *Candida* spp and (ITS) region of *C. albicans* genes were designed on the basis of published paper. The PCR products were run on (1.2%) agarose gel (Promega, USA) and electrophoreses were performed at 100 V in TBE buffer. The gel was pre-stained with 0.05% ethidium bromide (PromegaUSA). The gel was exposed to UV using UV light transilluminator and then photographed using digital camera (Sony-Japan).

Statistical Analysis

The statistical analysis performed with statistical package for social sciences (SPSS) 19.0 and Microsoft Excel 2010. **Table (1) primer of *Candida* spp and *Candida albicans***

Finding

Isolation and Diagnosis *Candida* species

Out of 50 samples, 60 isolates were identified among oral swabs of patients (men, women and babies) who suffered from oral candidiasis. 50 (83 %) contained only species of *Candida* and 10 (17%) contained more than one species *Candida*. The prevalence of different species of *Candida* was as follows: *C. albicans* (33%), The other identified species were *C. krusi* (17%), *C. tropicalis* (13%), *C. glabrata* (10%), *C. parapsilosis* (4%), *C. dubleniensis* (2%) respectively (Table.2,3) (Figuer.1). Among men patient with oral candidiasis, 84% were infected with one species of *Candida* while 16 % were infected with more than one species. Of those women patients 80% were infected with one species compared to 20 % who were infected with more. Babies show signal infection with *C. albicans* only (100%). There was statistically significant difference between these groups ($p < 0.05$). The prevalence of the isolated species among patients is shown in (Table.3).

Table (2) Germ tube, Chlamydospore, API 20 and Colony color of oral *Candida* spp

Species	No.of isolates	Germ tube (%)	Chlamydospore (%)	API 20 AUX Kit	Colony color on CHROMagar and texture
<i>C. albicans</i>	20(33%)	18(90%)	17(85%)	20(100%)	Light green and smooth
<i>C. krusi</i>	10(17%)	0	0	10(100%)	White cream
<i>C. tropicalis</i>	8(13%)	0	0	8(100%)	Blue-pink
<i>C. glabrata</i>	6(10%)	0	0	6(100%)	White –pink with white border
<i>C. parapsilosis</i>	4(7%)	0	0	4(100%)	Pink to cream
<i>C. dubleniensis</i>	2 (3%)	0	0	2(100%)	Dark green and rough

**Fig.1 (A) *C.albicans* by API 20canda -AUX kit,(B) *C.albicans* by CHROMagar****Table (3) prevalence of *Candida* spp among patients target**

Candida Species	Women	men	Babies	Total
<i>C. albicans</i>	10	7	3	20(33%)
<i>C. krusi</i>	5	4	1	10(17%)
<i>C. tropicalis</i>	4	4	0	8(13%)
<i>C. glabrata</i>	4	2	0	6(10%)
<i>C. parapsilosis</i>	0	4	0	4(7%)
<i>C. dubleniensis</i>	1	0	1	2 (3%)
<i>C. albicans</i> + <i>C. glabrata</i>	4	2	0	6(10%)
<i>C. albicans</i> + <i>C. krusi</i>	2	2	0	4(7%)
Total	30 (50%)	(42 %)25	8%) 5	60 (100%)

Molecular diagnosis by PCR

Results showed all (50) samples patients had positive results by molecular and conventional method. In this study, it has been found that primer pair CANIA and CANIB were successful in amplification of target region of 18S rRNA gene for *Candida* spp. with a PCR product with a size of approximately 210 bp (Figure.2). Concerning specific primer pair for *C. albicans* CALB1 and CALB2, it yielded approximately amplification size of 273-280 bp, but it did not give in amplification products with non-albicans species like lane B and lane I (Figure. 3). The amplification sizes of this study are alike with the results of subsequent studies that was 18S rRNA gene generated amplification size of 210 bp while (ITS) region gene produced amplification size of approximately 273 bp.



Fig.(2) Agarose gel electrophoresis of amplified PCR products for *Candida* isolates by universal primer CANIA and CANIB, lane A 100 pb. Molecular size marker, lane B-M represent *Candida* isolate.



Fig.(3) Agarose gel electrophoresis of amplified PCR products for *Candida* isolates by specific primer CAIB1 and CALB2, lane A 100 pb. Molecular size marker, lanes C-H, J-M represent *C. albicans*, B and I non *C. albicans*

Effect predisposing Factors on Oral Candidiasis Infection

There are many Predisposing Factors for developing Oral candidiasis infection such as Denture wearing, Smoking, use of certain drugs like: antibiotics, Contraceptive pills, or anything with cortisone or steroids, Breast thrush of mother, fatness, diabetes, Immunocompromised, meal with high sugar and yeast content, or tiny in vitamins and minerals. In this study the patients women are, 40 % wearing dentures, 24% Antibiotic utilization, 16% diabetic, 16% immunosuppressant, 4 % eating diet with high sugar content. Of men patients there are 35 % wearing dentures, 10 % used broad spectrum antibiotics, 15%

smoking, 20% diabetic, 20 % immunosuppressant. While in baby 100% Breast thrush infection (mother milk) (Table.4).

Table (4) predisposing Factors on Oral Candidiasis Infection

Factor	Women	Men	Babies
Wearing dentures	10 (40%)	7(35%)	0
Smoking	0	3(15%)	0
Diabetes mellitus	4(16%)	4(20%)	0
Immunosuppressant	4 (16%)	4(20%)	0
Antibiotic utilization	6(24%)	2 (10%)	0
Breast thrush infection (mother milk)	0	0	5(100%)
Diet with high sugar content	1(4%)	0	0
Total	25 (100%)	20(100%)	5 (100%)

Dissection

The findings of current study are consistent with the results of [13], they reported that *C. albicans* was the most common isolated species with prevalence 47% in women suffering from oral candidiasis and *C. tropicalis* the second most common isolated species with a prevalence of 32% then *C. dubliniensis* 28% and *C. glabrata* 12% and *C. krusei* 11%. Respectively. *C. albicans* is the species most frequently causes superficial and invasive infection at different anatomical sites in patients all over the world. It has a well known pathogenic potential and its main pathogenicity and virulence factors, are capacity to adhere to different mucosa and epithelia, dimorphism, with production of pseudohyphae helping tissue invasion, thermotolerance, and exoenzymes like proteinase and phospholipase and germ tube formation with consequent development of the filamentous form [14]. The mannan (glycoprotein present on the cell surface of *C. albicans*), adhesion responsible for the attachment of *C. albicans* to host cells are stronger than *C. tropicalis* and *C. prapsilosis* [15]. The prevalence of other species had the same pattern in

present study compare to earlier findings, The prevalence of non- albicans species seems to increase steadily and that raises the concern regarding the increase of anti-fungal drugs resistance ^[16]. The Molecular results are in line with that of ^{[11],[12]}. The PCR results agree with to the phenotypic patterns in table 2; the specific gene do not amplify the target DNA of non-albicans isolates; at the same time, these specific gene amplified the target DNA of *C. tropicalis* which revealed a blue color. “This type of contradictory diagnosis by CHROMagar when compared with molecular diagnosis may show that CHROMagar was not always essential for presumptive diagnosis of *Candida* species. So there was required arrangement of some phenotypic and molecular methods for presumptive identification of most *Candida* isolates. These results are the same the ones obtained by ^[17] who stated the limitation of phenotypic tests and the molecular methods, especially PCR which was being increasingly used for rapid detection of *Candida* than conventional phenotypic methods”, has become an emerging problem that deserves more clinical attention. Denture wearing, and poor denture hygiene, particularly wearing the denture continuously rather than removing them during sleep ^[18]. Is another risk factor, both for candidal carriage and for oral candidiasis. Dentures provide a relative acidic, moist and anaerobic environment because the mucosa covered by the denture is sheltered from oxygen and saliva ^[19]. Loose, poorly fitting dentures may also cause minor trauma to the mucosa, which is thought to increase the permeability of the mucosa and increase the ability of *C. albicans* to invade the tissues ^{[19],[20]}. The findings of this study are consistent with the results of ^[21], who reported that risk factors as overweight, diabetic, breast infection, used antibiotic drugs increasing fungal infections in women. The host defenses against *Candida* infection include T. cell immunity to prevent colonization and superficial invasion and phagocytic immunity to prevent deeper tissue invasion and hematogenous dissemination. These conditions that suppress any of these arms of the immune system include premature neonates severe burns, cancer especially hematologic malignancies, AIDS or immunosuppressive therapy such as steroids or cancer chemotherapy and organ transplantation especially liver transplantation ^[22]. The prolong use of antibiotics is a common cause of candidiasis. Antibiotics destroy both harmful bacteria and good bacteria. When antibiotics destroy friendly bacteria, it gives the *Candida* a chance to begin to multiply^{[23],[24]}.

Conclusion

PCR method confirmed the diagnosis for *Candida* isolates more than culture on CHROMagar medium and other phenotypic technique. Oral Candidiasis may be opportunistic infection among healthy persons and patients.

Conflict of Interest: None

Ethical Clearance: Oral approval from Patients

Source of Funding: Self

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Relationship between Smoking and Some Common Diseases and Health Programming to Quit Smoking in Technical Institute in Mosul - Iraq

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Abstract

Background smoking is important factor which play in development many lethal disease for human such as lung diseases ; ulcers; cancer of the tongue; larynx ; tongue ,therefore aim of current study to determining the extent to which smoking causes some specific cases to emerge. Material and Method This study has been done upon (266) male teachers in Technical institute in Mosul their age ranged between 21- 65 years. Result current study found that 107 (40.2%) were smokers and 159(59.8%) of the sample were non smokers . Also study showed that gingivitis was most disease occurs among smokers (55%) then peptic ulcer (53%) ,Anaemia (50%) , hypertension (48%) and allergy (44%) respectively, Also the study showed that number of smokers who try to stop smoking was 70(65%) and who stop then returned to smoke 62(57.9%) . Number of smokers who know negative results of passive smoking was 91(85%) from smokers and 36(85.5%) from non – smokers .95 smokers (91.5%) and 149 non- smokers was think that smoking is harm in general. Conclusions Most smokers were young men , So spread of the smoking phenomenon among the productive groups in the society was indicated . Our present study found that gingivitis was higher in smokers (55%) than non smokers (45%) ,gastric ulcer in smokers (53%) and (47%) in those who didn't smokers, as well as no instructions and programmes in curricula that recognize the harms of smoking,

Keywords: *smoking ; health programming ; quit smoking.*

Introduction

Smoking is a very bad habit , and rising among pupils and youth all over the world especially in Arab countries like Yemen .Smoking also has negative results wards allowing little boys to smoke in the future [1]. Smoking is still one of the most causes of different diseases all over the world , so number of smokers now is about billion persons especially in comity from low and middle income countries. Smokers facing several problems like ,lung diseases ,heart diseases. In spite of awareness of media about negative effect of smoking , but the habit still common among teenage group [2,3]. There are high percentage of smoker in Saudi Arabia nearly 45% among adult meals , Kingdom spend about (5) billion riyals every year (1.3) billion US \$ for tobacco use [4]. Everyone knows that smoking is a bad habit, and most of the people in America believe that smoking is a must, however, it remains documented as one of the health problems in the world [5].

Gingivitis in smokers leads to grow many types of pathogenic bacteria in saliva with lower GBI than non – smokers means there are a bad effect of smoking on immunity of human body [6]. Anaemia has negative result on quality of life ,and it was significantly associated with lung diseases ,dyspnea ,level of systemic inflammation signs and the smoking index [7]. There were correlation between smoking and many chronic respiratory diseases such as bronchitis , emphysema ,asthma or more than one by using pulmometer tests and other instruments [8]. Less of gingival bleeding in smokers has been associated with decrease of density of blood vessels . The mechanism of that effect is not precisely defined , but there were relationship between VEGF expression mast cell count and period of smoking [9].

The worst results are those among prisoners, whereby many of them cannot give up this habit and the effect of nicotine was vivid among them because the

nicotine molecules are connected with other compounds inside the body [10].

Smoking has many negative effects on body function, so in youth to middle age persons there were increase in arterial blood pressure (Diastolic blood pressure) according to smoking status means non – smokers, light smokers and heavy smokers [11]. The effect of smoking on blood pressure in most cases is limited, yet the effect of some substances existing in tobacco has a genic impact on some people, that would lead to disorders in blood pressure levels especially when drinking alcohol together with smoking [12]. Some studies found relationship between cigarette smoking and allergy in active smokers and secondhand smoke, and there were about half of population had detectable levels of IgE specific to at least one inhaled allergy [13]. Smoking is risk factor for organ system diseases. Control program against smoking must include banning smoking in public places, anti – smoking media campaigns targeted towards children, community, school and college and encouraging smokers to quit [14].

In spite of many efforts that were exerted to stop smoking in Viet Nam, a study made by one of the groups specialized in smoking among adult males, revealed that there is still nearly half of the individuals of the sample are smokers [15]. It is very difficult to help heavy smokers to stop, that because they start smoking as children or teenagers [16].

The study aims to find relationship between smoking and gingivitis, peptic ulcer, hypertension, anaemia and respiratory allergy.

Material and Method

A questionnaire form was prepared including specific data for the randomly selected sample handed out to (266) male employees at the departments and units of the Technical Institute/ Mosul-Iraq. After collecting the forms the results were analyzed and compared between a sample of smokers whose number was (107) and another of non-smokers whose number was (159).

Findings

Table(1): Distribution of smokers and non smokers according to age group.

Non smoker	Smoker	Age (Years)		
%	No.	%	No.	
39%	62	44.8%	48	21-40
(20.8%)	33	35.6%	38	41-50
40.2%	64	19.6%	21	51-65
59.8%	159	40.2%	107	Total

The study showed that number of smokers of the sample individuals was 107(40.2%) and non-smokers was 159 (59.8) of the whole group number which is (266) male employees whose age ranged from (21-65) years. The highest rate of smokers was in the (21-40) group and their number was 48 (44.8%), followed by the (41-50) group and the number was 38 (35.6%), and finally the (51-65) group with the number 21 (19.6%). As for the non-smokers group, the (51-65) group was the largest in which the number was 64 (40.2 %) of the non-smokers group total number (159), then the (21-40) group with the number 62 (39%), as for group (41-50) it was the least number of non-Smokers which was 33 ((20.8%) .

Table (2) Types of disease in both smoker and non smoker

Total	Non smoker	Smoker	Types of disease		
	%	No.	%	No.	
34	47%	16	53%	18	Gastric ulcer
60	45%	27	55%	33	Gingivitis
32	50%	16	50%	16	Anaemia
85	52%	44	48%	41	Hypertension
77	56%	43	44%	34	Respiratory allergy

Table (2) illustrates some of the cases and the rate of getting infected with diseases among both smokers and non-smokers. Among smokers the rate of getting infected with high blood pressure reached 41 (48%) and

non-smokers 44 (52%) and those with allergy-among smokers- reached 34 (44%) and 43 (56%) non-smokers, then those suffering from gingivitis 33 (55%) of smokers and 27 (45%) of non-smokers, and the number of those

suffering from gastric ulcer of smokers was 18 (53%) and of non-smokers was 16 (47%), and the number of those suffering from anaemia among smokers was 16 (56%) and non-smokers 16 (56%).

Table (3) Relationship between disease and period of smoking

Total	period of smoking (years)	Types of disease			
	10-35	3-9			
	%	No.	%	No.	
18	83%	15	17%	3	Gastric ulcer
33	93%	31	7%	2	Gingivitis
16	68%	11	32%	5	Anaemia
41	92%	38	8%	3	Hypertension
34	82%	28	18%	6	Respiratory allergy

Table (3) showed the relationship between the period of smoking and getting infected with diseases- the present study, smokers were divided, according to period, into a group of (3-9) years and another of (10-35) years. The study has found that the number of those suffering from ulcer of the first group (3-9) years was 3 (17%) and of the second group (10-35) years was 15 (83%) of the total smokers infected with this disease.

As for the number of those with gingivitis was 2 (7%) of the first group and 31 (93%) of the second group, the number of those suffering from anaemia of group one was 5 (32%) and group two was 11 (68%). It was also shown that the number of those with high blood pressure of the first group was 3 (8%) and 38 (92%) of the second, as for the allergy in the respiratory system, the first group recorded 6 (18%) and the second one recorded 28 (82%).

Table (4) comparative Behavioural aspect and guidance education between smoker and non- smoker.

Non smoker	Smoker	Behavioural aspect and guidance education		
%	No.	%	No.	
—	—	65%	70	Try to stop smoking
—	—	57.9%	62	Try to stop smoking and returned
85.5%	136	85%	91	Known harm of passive smoking
93.7%	149	91.5%	98	Think about general risk of smoking
79.8%	127	74.7%	80	Eat fruit and vegetables daily
66%	105	68%	73	Any guides posters in establishment
49%	78	55%	59	Conducts guidance symposiums by establishment

Concerning the behavioural aspect of smokers, result showed that 70 (65%) of the smokers tried to quit smoking but couldn't and 62 (57%) of them stopped smoking then returned to smoke. Concerning health education among smokers, the study showed that 91 (85%) of them were know the harm caused by passive smoking on others, while the number of those non-smokers who know the harm of the passive smoking was 136 (85.5%), and 98 (91.5%) of smokers believe that smoking is a bad habit in general, and 149 (93.7%) of non-smokers. When the sample individuals were asked about the nutritional status represented by eating vegetables and fruit on a daily scale, the answers were 80 (74.7%) of smokers positive and the number of those non-smokers who care for nutrition was 127 (79.8%).

As for health and guidance education available at the institution units and department under study, 73 (68.2%) of smokers answered that there were, at their departments and units, guiding stickers about the bad effects of smoking, and the number of non-smokers was 105 (66%). About the activities of the establishment, 59 (55%) of the smokers answered that the establishment conducts guidance symposiums about the harms of smoking. Table(4).

Discussion

The most common diseases among smokers are gingivitis, and front teeth inflammation, the cause for that is the existence of one kind of bacteria in the saliva of smokers as was emphasized by a similar study [17,18] Peptic ulcer is one of disease affected by some risk factors .several studies found significant correlation between peptic ulcer and smoking ,some drugs ,drinking and age [19]. Present study shows that getting infected with high blood pressure has a partial relation with smoking addiction whereby the number of those suffering from disorders in blood pressure levels in the sample under study of non-smokers was more than that of smokers and that is an indicator that there is no direct relationship between this disease and smoking, but, there is a negative impact of smoking on some factors such as cholesterol molecules which cause hypertension and that was asserted by international researches [20].

The number of those infected with anaemia was the same among smokers and non-smokers and the reason to their infection with this disease may be attributed to other causes, perhaps pathological cause, we believe

that the reasons for disease are more likely and that is due to the fact that all the individuals of the sample are of those who care for the nutritional aspect and eat vegetables and fruit on a daily scale.

The present study has showed that half of the smokers had stopped smoking then returned practising this habit the thing that signals that there is not enough preparation to get rid of smoking especially most of them were addicted to smoke for many years and the cause may be that there wasn't enough health guidance and instructions to prohibit smoking at the establishment. The smoker who tried to get rid of smoking may not find the encouraging environment against smoking because of the dirtiness of public places and streets, the spread of engines smoke and transportation and others. This means that there is no public systems and education in the society that tend towards clean environment and rid it from pollutants and self-commitment with public cleanness. Many countries have pushed forward towards realizing an environment clear from smoke which is the sole way to be sure to protect the health of peoples. These countries have committed most of the closed work places and public places to be clear of smoke (100%). These measures have emphasized the resulting health benefits of preparing clear environments which proved positive and the possibility of application at all levels [21]. Getting infected with allergy among non-smokers was more than among smokers, however, the cause was that the respiratory system of smokers has become used to smoke for long periods and their smell sense has become saturated to the contrary of non-smokers who sense smoke more than others.

Conclusions

Smoking is still a bad habit among teachers and other workers in the establishment and many smokers have spent a lot of time and cannot quit so easily and most smokers were 21 to 40 years of age, and this is an indicator for the spread of the smoking phenomenon among the productive groups in the society. Our present study found that gingivitis was higher in smokers (55%) than non smokers (45%) ,gastric ulcer in smokers (53%) and (47%) in those who didn't smoke but in anaemia case we found same percentage in both groups (50%) may be because another reason such as mal nutrition ,parasites ,we found also respiratory allergy and hypertension in non – smoker were higher (56%,44%) and (52% ,48%) respectively. And about health programs we found no

public system and education in the society that tend towards clean environment and rid it from pollutants and self – commitment with public cleanness.

Conflict of Interest : Nil

Source of Funding : Self

Ethical Clearance : Not required

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Epidemiological Analysis and Evaluation of the Effectiveness of the Mumps Surveillance Programme Adopted in Babylon Province during the Year 2017

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Abstract

Background: The outbreaks of the wild mumps virus continues in certain parts of the world despite the wide application of immunization programs. **Objectives:** The goal of the study to determine the factors that cause an outbreak of mumps and low averages of vaccination, to assess effectiveness and failure of a vaccine surveillance program in Babylon governorate / Iraq during the year 2017. **Method:** This study was performed in all health centers of the Public Health Directorate in the governorate, through mumps cases recorded and reported by the Directorate during the period January 1, 2017 to December 31, 2017, After that has been statistic and evaluate the performance of monitoring the vaccination program in accordance with the principles of the WHO in Iraq and the world. **The findings:** Of the 3176 suspected cases, 1864 (58.69%) confirmed cases of mumps. The results showed that people aged <1-10 years were the most vulnerable about 612 (80.74%). Mumps incidence in rural areas was higher than urban areas in the Babylon province {954 (63.68%), 910 (54.23)} respectively, male casualty percentage 1375 (64.40%) topmost of the female 489 (46.97%), and also, people immunized with one dose of the vaccine at least were less susceptible to mumps compared to people who did not vaccinate, which was 968 (54.57%) and 661 (64.43%), respectively. **Conclusions:** Mumps remain an important cause of the disease in the Babylon province / Iraq. Proper vaccination coverage as recommended by WHO, as well as rapid reporting on all cases of suspected are all important factors for the elimination of infection in Iraq and the Babylon province in particular.

Keywords: Mumps virus; Epidemiology analysis; Surveillance the vaccination program in Babylon / Iraq

Introduction

Mumps is an acute viral disease characterized by the tenderness of uni- or bi-lateral or parotid swelling or other salivary glands. Mumps disease is transmitted by contact from person to another or direct connection with the respiratory system of spray or saliva from an infected individual ^[1, 2]. Mumps virus multiplies in the nasal pharynx and lymph nodes, with secondary viremia, occurs late in the incubation period. During those 3-5 days of viremia, the virus is spreading in the main target organs. Although salivary glands are the most commonly affected, it can also share the central nervous system, pancreas, liver, spleen, kidneys and reproductive organs, the average period of incubation is between 16-18 days ^[3].

The clinical presentation ranges from without apparent symptoms of infection, mainly respiratory symptoms, to complications with or without parotitis. Parotiditis is a widespread manifestation, occurring in approximately 60% to 70% of mumps infections, but can range between 50% and 95% depending on age and population immunity. Parotiditis typically lasts seven to ten days, and maybe initially unilateral, but becomes bilateral in about 65% of cases. Symptoms of prodromal are non-specialized, including of a headache, anorexia, vomiting, malaise, myalgia as well as low-grade fever. Invisible or non-diagnostic infection could occur among children, especially at school age ^[4, 5].

Mumps complications may vary by age and sex, it can occur without parotitis. Serious complications, including deaths, are rare. Complications involving CNS, in the form of sterile meningitis, they are common. Meningitis is mostly benign and dissolves without succession. Asymptomatic meningitis occurs in up to 55% of patients in studies, where the lumbar punctures are routinely performed, however, clinical symptoms that indicate meningitis at 0.02% - 10% of Parotiditis cases. Encephalitis occurs in 2-4 per 1000 cases of mumps and can be fatal [5, 6, 7].

In males, testicle inflammation (Orchitis) is more common complications, occurs in about 30% of men after puberty (range: 19% - 44%). In some cases, up the degree of testicular atrophy, but infertility is rare. In postnatal women, mastitis occurs in up to 30% and ovarian inflammation (Oophoritis) in about 5% of cases[7].

The least common complications include pancreatitis, deafness, myocarditis, arthralgias, cerebellar ataxia, ascending polyradiculitis, thyroiditis, thrombocytopenia, arthritis, transverse myelitis, nephritis and endocardial fibroelastosis [8]. **The aim of the current study is to find out** the reasons associated with this outbreak of mumps and low coverage of vaccination, to assess effectiveness and failure of a vaccine surveillance program in the Babylon province / Iraq during the year 2017.

Material and Method

Across-sectional study was performed in 147 health centers of the Public Health Directorate in the governorate, through Mumps surveillance data recorded and reported by the Directorate during a period from January 1, 2017 to December 31, 2017. Permits

and moral approvals obtained from Public Health Department dependent to Ministry of Health in the governorate, where data were collected during a period from January 3, 2018 to March 15, 2018 according to the clinical criteria of the disease (ICD-10-B26).

The study included through the monitoring data of the patient's sex, age, and resident and vaccination status in addition to the investigation of the data, where the status of immunization was determined through the patient's oral report or a review of its own immunization card. After that, all monitoring enters data into Microsoft Excel 2013 program (USA) so that we can calculate the variables for each variable according to the study (Months, gender, and age, geographical distribution, and vaccination status) for individuals who have confirmed mumps condition.

Findings

A total of the 3176 suspected cases of mumps were notified in Babylon province/ Iraq from 1 January 2017 to 31 December 2017. 1864 confirmed cases of mumps in a laboratory. Table (1,2,3,4,5) showed mumps of suspected and confirmed cases including months, sex, age group, geographically distributed as well as vaccination status. All notified cases were of Iraqi nationality and, fortunately, no mumps-related deaths or serious complications e.g., Orchitis (sterility) or meningitis were reported. Ages ranges of mumps suspected cases of 3 months to 73 years, while the cases were confirmed from (1> - <60) years. Table (1) showed the percentage of infection, according to the months of the year, the highest percentage was in April, January and May (82.99%, 85.94%, and 89.46%, respectively) and the lowest percentage in November (15.07%).

Table (1): Suspected and confirmed mumps cases according to months, Babylon province/ Iraq, during the year 2017

No.	Months	Suspected cases	Confirmed cases	Infection ratio %
1	January	320	275	85.94
2	February	414	271	65.46
3	March	311	214	68.81
4	April	435	361	82.99

Cont... Table (1): Suspected and confirmed mumps cases according to months, Babylon province/ Iraq, during the year 2017

5	May	332	297	89.46
6	June	189	105	55.56
7	July	233	83	35.62
8	August	218	43	19.72
9	September	245	65	26.53
10	October	123	52	42.28
11	November	146	22	15.07
12	December	210	76	36.19
Total		3176	1864	58.69

Mumps remains one of the dominant causes in Iraq and the province of Babylon in particular. Mumps disease tends to cluster during spring and winter while declining at end of the summer (Table 1). This is a typical seasonal mumps outbreak in moderate climates. Probably due to these outbreaks the gathering of children in school, people gathering at home, workplaces during cold weather, and environmental factors conducive to virus transmission ^[9].

Table (1) showed increase in confirmed mumps during the months of the year. There are factors number

that can explain the phenomenon, including low vaccination coverage rates and the high failure rate of the vaccine. The mumps vaccine rate observed in Iraq was exceptionally high, although it was less than the failure rates observed during recent outbreaks in neighboring countries ^[10, 11].

Table (2) shows the percentage of male and female infection in suspected and confirmed cases in the province, with the highest percentage of males (64.40%) compared with females (46.97%). This finding consistent with ^[12, 9, 13].

Table (2): Suspected and confirmed mumps cases according to gender, Babylon province/ Iraq, during the year 2017

Sex	Suspected cases	Confirmed cases	Infection ratio %
Male	2135	1375	64.40
Female	1041	489	46.97

Where the ages of the infected less than one year to 73 years, and most of the infected in the age group (1>-10), (11-20), which was 80.74%, 72.03%, respectively and Table (3) illustrates this. This finding is an agreement with ^[14].

Table (3): Suspected and confirmed mumps cases according to age, Babylon province/ Iraq, during the year 2017

Age group (years)	Male			Female			Total		
	S.c.*	C.c.**	%	S.c.	C.c.	%	S.c.	C.c.	%
1<-10	512	435	84.96	246	177	71.95	758	612	80.74
11-20	943	710	75.29	337	212	62.91	1280	922	72.03
21-30	334	160	47.90	145	78	53.79	479	238	49.69
31-40	156	39	25.00	124	14	11.29	280	53	18.93
41-50	88	24	27.27	122	6	4.92	210	30	14.29
51->60	102	7	6.86	67	2	2.99	169	9	5.33

* Suspected cases, ** Confirmed cases

Low vaccine, the potential in older age groups (Table 3) has been explained by fact that most of them were born prior to the adoption of the program of immunization in Iraq in the eighties or through first years of routine vaccination against mumps when lower coverage rates characteristically [15]. On the other hand, the high probability of vaccination among those aged 1>-10 years coincides with an average coverage ratio of 85% from 1995 to 2002.

Another notable finding in the present study is the high percentage of confirmed cases were found among these young children, this indicates the need for sufficient vaccine coverage for this age group.

The data in a table (4) showed that Suspected and confirmed mumps cases, according to the geographic district. As it appeared, rural is higher than urban (63.68% and 54.23%, respectively), these results agree with previous studies by [9].

Table (4): Suspected and confirmed mumps cases according to the geographic district, Babylon province/ Iraq, during the year 2017

Geographic district	Suspected cases	Confirmed cases	Infection ratio %
Rural	1498	954	63.68
Urban	1678	910	54.23

The analysis also revealed that cases in rural areas of the province are more likely to be mumps. This theory is apparently contradictory to the common idea of higher rates of mumps in urban areas. With regard to the performance monitoring system Mumps in Babylon / Iraq province through the study period, data analysis revealed that indicators exceeded the level of WHO [16].

The results of according to vaccination status of mumps are described in the table (5). The results revealed that 54.57% who were vaccinated for mumps have confirmed cases compared to non-vaccinated and unknown (64.43% and 62.32%, respectively).

Table (5): Suspected and confirmed mumps cases according to vaccination status, Babylon province/ Iraq, during year 2017

Vaccination status	Suspected cases	Confirmed cases	Infection ratio %
Vaccinated	1774	968	54.57
Non-vaccinated	1057	681	64.43
Unknown	345	215	62.32

The rate of vaccine failure is increased through factors such as dealing with an inappropriate vaccine, cold-chain maintenance failure and possibly other factors related to the host and the vaccine. Nevertheless, the vaccine effectiveness was 54.57%, it represents the vaccination rate in the entire population who developed defensive immunity [17, 18].

The failure to vaccinate people at risk remains an important reason for the adequate levels are lacking community immunity. Low rates of vaccine coverage can be due to resources are insufficient, hard to reach (residents of insecure regions, travelers, IDP families), lack of clear political will to vaccinate and fear of real or perceived negative events [19].

Finally, there was not enough data available for the single and booster dose. Since was rejecting cases may not represent the total population and the possibilities in this study may reflect the probability of disease confirmed for reporting cases.

Conclusion

We conclude that Mumps remains an important cause of the disease in the Babylon province / Iraq. Also, the percentage in January, April, and May were higher than in other months. In addition to the incidence of infection among males is higher than females, the incidence rate of age group less than one year to 20 years is higher than other groups, the incidence of infection in rural areas is higher than urban, and the proportion of non-vaccinated people were higher than vaccinated people.

Therefore, we recommended that more efforts be made to eradicate mumps, as well as expanding the coverage of vaccines and raising awareness of the

population with emphasis on the immediate notification of any case of people working in this area, on the other hand, give a booster dose and carry out periodic surveillance of individual antibody assessment to reduce the spread of epidemics, in addition to conducting serological and molecular studies to determine the causes of the failure of the vaccine as well as identify common strains to determine outbreaks.

Conflict of Interest: None declared.

Source of Funding: Ministry of Health / Babylon Health Directorate / Public Health Department.

Ethical Clearance: It is a pleasure to thank the employees of the Public Health Department/ Babylon to help me to get the study subjects

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Histological and Fluorescent Microscope Studies for Evaluation of Carbon Accumulation in Lymphatic tissues in Birds within Polluted Areas (*Anas platyrhynchos*)

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Abstract

In order to follow up the deposition of carbon in the lymphatic tissues of birds, 18 birds of the ducks were collected. Divided into three groups of (6) birds per group. All the lymphatic tissues of the cecal tonsils and the lymph node in the lung of the birds of polluted areas contain the carbon aggregations in a variable manner. Carbon aggregations were slightly higher in the lymphatic tissue collected from brick factories polluted areas than those collected from oil fields polluted area. The effect of carbon aggregations on all lymphatic tissue cells was evident in this study, which caused the death of these cells (programmed death), which is clearly demonstrated by using AO / EB double stain microscope technique, where normal cells appeared green, and other cells appeared in yellow color which indicates early apoptosis, while other cells appear orange as an indicator of the late stage of cell death.

Key words: Carbon, Acridine orange/Ethiedium bromide, Fluorescent microscope, cecal tonsils, lung lymphoid nodes.

Introduction

The best-studied class of organism are birds, and various investigations have shown their significance as important mobile linkups in the dynamics of natural and human-dominated ecosystems^[1]. The lymphatic system includes lymphoid organs like the lymph nodes, spleen, tonsils, Peyer's patches, and thymus, all of which play an significant role in the immune response^[2]. Tonsils are secondary lymphoid organs^[3]. They are an aggregation of enormous lymphoid nodules, located in the lamina propria and the sub macosa of the cecum^[4]. They are close to the ceca openings, at the limit between ileum and colon, in the ceca walls, from a histological point of view, the tonsils are covered with a stratified epithelium associated to the follicles. In the cecum wall, in the ilocecal ligament insertion point there is diffuse lymphoid tissue in the mucous membrane, in geese there are nodules with obvious lymphatic sinus in the cecum mucous depth and even in its muscular^{[5][6]}.

In conjunction with the urbanization, brick industries are rapidly increasing and migrations of people into the city areas pressurize to produce more brick, as a result, more and more brick kilns are being built, at present, over 400,000 people are engaged in work in the brick industry^{[7][8][9]}. Brick manufacturing plants are the most polluted workplaces causing respiratory, gastrointestinal, reproductive, psychosocial and skin diseases. In regions around the world, oil is commonly categorized as one of the highest valued resources an organization can extract from the ground^{[10][11]}. Clearly, the world has a heavy dependence on oil, in addition, the economies of many countries^[12].

Oil contamination from drilling processes, however, has adversely affected the people living in these areas by polluting the environment around them, the water, soil and air have been severely tainted by petroleum pollutants, as a result, wildlife, livestock, and humans have been sickened^{[13][14][15]}. Globally, it is difficult to estimate how many people die off prematurely or get sick due to air pollution because people are exposed to

so many different pollutants in various concentrations over their lifetimes. However, according to WHO for air pollution approximately 3 million people have died each year. Among them, 800,000 people die prematurely every year due to lung cancer, cardiovascular and respiratory diseases, which are caused by outdoor air pollution [16][17][18]. Hurtig and San Sebastián conducted a study to contrast the cancer rates of people living near oil pits to people who live far from them, their research shows that people who lived in close proximity to the contamination had elevated rates of cancer of the stomach, rectum, skin, soft tissue, kidney, cervix, and lymph nodes, they also found a great increase in hematopoietic cancers among children who were less than 10 years of age. Studies in animals have shown that ingestion of carbon tetrachloride increases the risk of liver cancer, EPA has classified carbon tetrachloride as a Group B2, probable human carcinogen [19][20][21]. The liver and kidney are target organs for carbon tetrachloride toxicity, moderate irritation after dermal application was seen on the skins of rabbits and guinea-pigs, and there was a mild reaction after application into the rabbit eye [22][23]. Carbon monoxide (CO) is a nonirritating colorless, odorless gas produced by incomplete burning of carbon containing fossil fuels. The normal concentration in atmosphere is less than 0.001% and a concentration of 0.1% can be lethal[24]. Mild exposures result in headache, malagia, dizziness, or neuropsychological impairment [25][26]. Severe exposures to carbon monoxide result in confusion, loss of consciousness, or death [27]. It causes thousands of uncalled for deaths each year. Patients who survive the initial poisoning can develop delayed neurologic dysfunction, which occurs in 14-40% of serious cases[28].

Aim of this Study:

The present histological and ultrastructural studies were undertaken mainly for two reasons:

(1) To attempt to confirm the finding, obtained by light and electron microscope, that carbon particle aggregation through the lymphatic tissues and its incidence on birds which living in the soot polluted area.

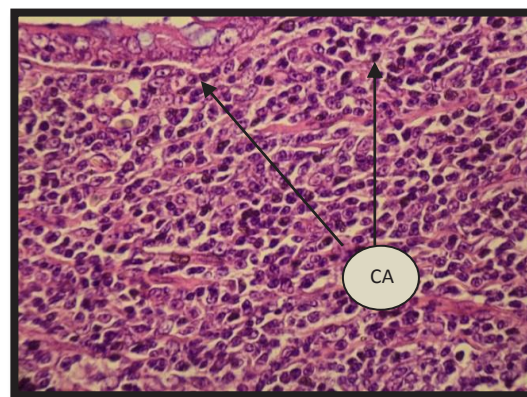
(2) In order to evaluate the role of effect of brick manufactories and oil fields on poultry farming comparing to the other healthy bird farms.

Materials and Method

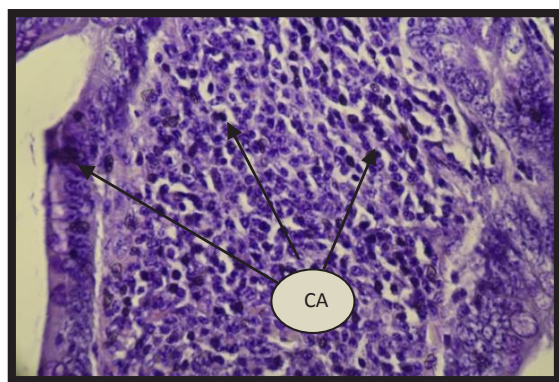
Eighteen birds of domestic ducks were collected from different healthy and polluted areas of Wasit city, the birds were divided according to the area collected from into three groups (Al-Dijaili area, AlAhdab oil field area, and brick factory area) each group were six birds, all birds should be clinically healthy and devoid of any type of injuries. All domestic ducks were slaughtered and dissected and the cecal tonsils and lung associated lymphoid nodules samples were taken from the abdomen and thoracic region and were fixed in 10% formalin for (72) hours, then and there washed up in tap water for 2-3 hours and then moved the samples to numerous histological techniques as followed: dehydration, clearing, infiltration, embedding, cutting and staining with hematoxylin and eosin (H&E) stain, periodic acid sciff (PAS) stain for appearing the general structure of the cecal tonsils and lung associated lymphoid nodules and todetect aggregations of carbon in paraffin sections. In addition to acridine orange ethidium bromide (AO/EB) double stain to detect apoptosis and necrosis which occurred in affected lymphoid tissue.

Finding Discussion

The microscopic investigation with the H&E stain besides the PAS (periodic acid sciff) stain of the cecal tonsils histological sections showed that there are slightly carbon aggregations scattered in the lymphatic tissue found in the sub mucosa layer (Fig. 1,2).

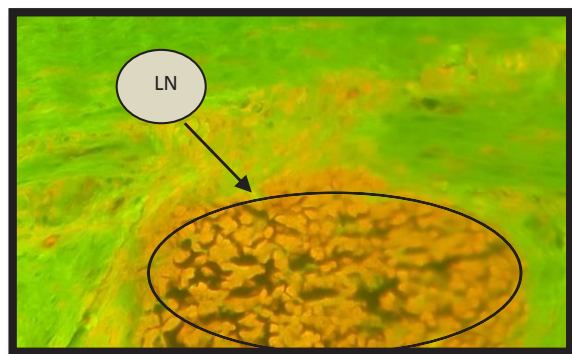


(Figure1): cross section of cecal tonsils of the domestic duck (oil fields) showing the scattered carbon aggregations (CA) (H&E stain, 100X).



(Figure 2): cross section of cecal tonsils of the domestic duck (brick factories) showing the scattered carbon aggregations (CA) (PAS stain, 100X).

Likewise, the cecal tonsils sections of polluted area samples were stained by acridine orange/ethidium bromide (AO/EB) double stain and examined with a fluorescence microscope. It showed that all the lymphatic tissue cells were under cell apoptosis, all cells appeared yellow (Fig. 3).

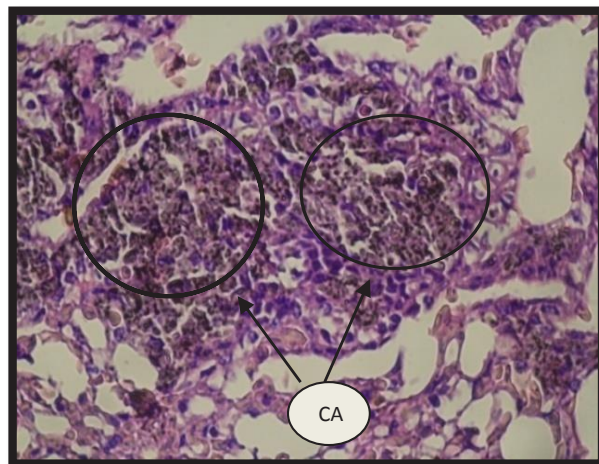


(Figure 3): Fluorescent microscope section of cecal tonsils of domestic duck (brick factories) showing the whole lymphatic nodule (LN) suffered from apoptosis in which the apoptotic cells appeared orange color (AO satin, 40X).

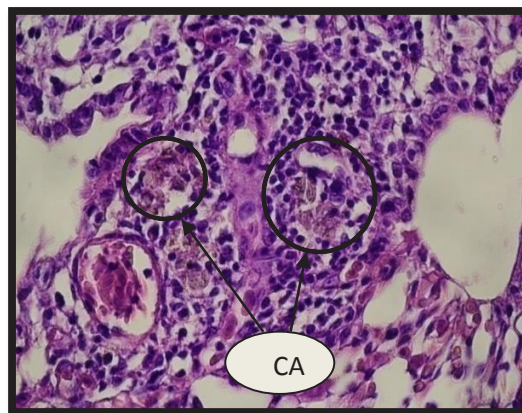
It is well known that soil has been severely tainted by petroleum pollutant, as a result, the carbon particles will enter the digestion system via contaminated food, and since the gut-associated lymphoid tissue is considered the first line of defense it will eventually accumulate there. These findings were configured out by [29][30][31].

On investigation of other lymphatic tissue in the affected ducks of the carbon polluted area, which lymphatic tissue in the lungs, the histological and histochemical examinations appeared black aggregations of carbon through lymph nodes of lung samples which collected from an area polluted by remnants of brick factories, whilst these carbon aggregations appeared relatively

minimal in lung lymphatic tissues of birds breeding near oil field (Fig. 4,5). The results were similar to those that observed by [32], in experimental animals which he mentioned that the carbon deposition during inhalation not only in the lung alveoli but also in the lung associated lymph node. Likewise, [33], which mentioned that there are minimal MWCNT depositions were observed in the peribronchial lymph nodes in the exposed group.

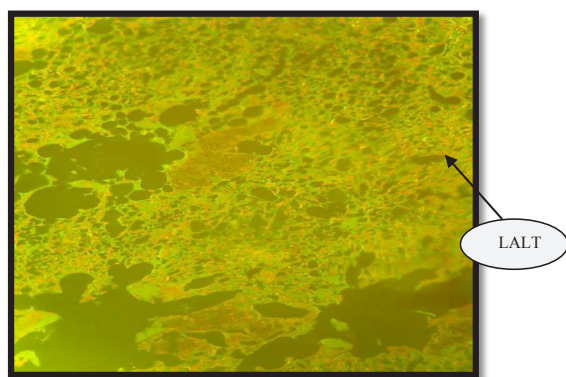


(Figure 4): Cross section of lung of domestic duck (brick factories) showing aggregation of carbon (CA) in the lung associated lymphatic nodule (H&E stain, 40X).



(Figure 5): Cross section of lung of domestic duck (oil fields) showing aggregation of carbon (CA) in the lung associated lymphoid tissue (H&E stain, 40X).

At the same time the sections of lung samples collected from polluted areas, and after treatment with acridine orange/ethidium bromide (AO/EB) double stain showed that there is apoptosis occurred and also cell necrosis, the lymphatic tissue cells were all in yellow and orange colored which is an indicator of DNA fragmentation occurred (Fig. 6).



(Figure 6): Fluorescent microscope section of lung of domestic duck (brick factories area) Showing lymphoid tissue (LT) undergo cell apoptosis in which the apoptotic cells appeared in orange and yellow color (AO/EB satin, 40X).

For the first time, we were able by histological and fluorescent technique to determine the gathering of carbon particles in the different lymphoid tissue of duck which raised in polluted poultry farms by factories and oil field remnants. Also noticing effectuation these particles on the cellular structure of lymphatic tissue and causing its death.

Source of Finding: Self

Ethical Clearance: Non

Conflict of interest: Non

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Hormones FSH , LH Relationship Polycystic Ovaries Syndrome(PCOS) and Delay Pregnancy in the Woman

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Abstract

Background: Polycystic ovary syndrome (PCOS) this is very important considered cause of endocrine disorders and infertility. One of the most important symptoms of this disease is the presence of large ovaries on the many and small cysts in both ovaries, and this are considered the most common reason for reproductive menstrual disturbance age]1[. the normal function for the reproductive endocrine include the wide range hormones controlled by complex feedback mechanisms . It is very important observation luteinizing hormone (LH) serum levels, follicle - stimulating hormone (FSH), follow a cyclical pattern closely coordinated by the hypothalamic - pituitary - gonadal axis, since LH, FSH, estradiol and progesterone play such a key role in the reproductive function of women.]2[.

Materials and Method: The purpose of this study is to identify the non-normal hormonal ratios for the LH and FSH in women with PCOS and evaluate this effect on the late the pregnancy. To assess effects of biochemical parameter TSH , prolactin and testosterone , and Compare them with healthy women .

Results: Most patients with (PCOS) will observe A total of symptoms by reason appear to be significantly higher in patients with PCOS than in controlled healthy in hormones such as luteinizing hormones (LH) , Follicle - stimulating hormone (FSH) , and Thyroid Stimulating Hormone (TSH) compare them with healthy results and in shows, there are no hormonal changes in testosterone and prolactin.

Conclusion: PCOS is one of the most common women's endocrine disorders in the age group between 18 - 44. It affects approximately 10% of women of childbearing age. It is one of the main causes of female fertility weakness, and the symptoms and severity of this syndrome vary considerably among affected women.

Keyword: *Polycystic ovary syndrome (PCOS), follicle-stimulating hormone (FSH), luteinizing hormones (LH).*

Introduction

Polycystic ovary syndrome (PCOS) is a common case of reproductive and metabolic dysfunction in women of reproductive age. Suggest diagnosed criterion for the (PCOS) contain the Two out of three characteristics: Excess androgen, menstrual irregularity and ultrasound polycystic ovaries (PCO). Moreover , PCOS in the women appearance Increased risk of type 2 diabetes and above spread of cardiovascular risk factors that appear to be linked to the classical phenotype]3[.

One of the main reasons to reduce the heart and metabolic risks in women with PCOS are there changes in the lifestyle , pharmacological and bariatric therapy. And shown the processing with using metformin was

appear to improve the sensitivity of insulin, decrease blood glucose levels and levels of androgen. There are more effects effective when associated in addition to reproductive abnormalities, (PCOS) was more powerfully associated with metabolic comorbidities in combination with lifestyle interventions, and most of them were linked to obesity . It can also be observed when standard diagnostic parameters are absent, clinical presentation heterogeneity and obesity presence make it very difficult to manage (PCOS). The approach to metabolic abnormalities should therefore be tailored to each woman's risks and treatment objectives]4[.

Hormone luteinizing (LH) and hormone stimulating follicles synergize during the condition (PCOS) and are produced in the pituitary gland of the brain by

gonadotropic cells. They play cardinal roles in puberty menstruation and fertility. Active rise of LH is known to triggers off ovulation. Luteinizing hormone and Follicle-stimulating hormone Hormones that promote ovulation. At the start of the cycle, levels of LH and FSH usually range from approximately 5 - 20 IU / ml. During the early part of their cycle, most women have about equal amounts of LH and FSH. However, LH increases in the amount of LH to about 25 - 40 IU / ml 24 hours before ovulation. Once the ovary releases the egg, the LH level falls back. Although many women with PCOS still have LH and FSH values within the range of 5 - 20 IU / ml, their LH level is often twice or three times that of FSH[5].

Follicle - stimulating hormone (FSH) plays a key role in ovarian follicular growth and development in female reproduction[6]. Both are considered luteinizing hormones and follicle - stimulating hormones, are members of a family of structurally similar glycoprotein hormones, which include pituitary thyroid - stimulating hormones and placental chorionic gonadotropin. Normal growth, sexual development and reproductive function depend on complex interactions between pituitary gonadotropins, gonadal steroid hormones. Each hormone consists of two different, non - covalently associated subunits, each of which is glycosylated, and is maintained in its proper tertiary structure by intra - chain disulfide bonds required for the assembly of subunits into active dimeric hormones [7]. On the other hand, the LH/FSH ratio is higher than the long-term “gold standard” in the diagnosis of PCOS, and it is also a fascinating problem recognizing the role of LH and possible associations with hyperinsulinemia and evaluating the usefulness of the gonadotropin ratio in the diagnosis of hyperinsulinemia PCOS, Meanwhile, essential diagnostic investigations in PCOS are still controversial[8]. And as well view Insulin resistance (IR) is one of the characteristic findings in patients with PCOS[9].

Materials and Method

In this study, we measured and compared hormonal values in women with polycystic ovaries and compared them with healthy subjects. The samples were collected from women in the morning and on the third day of the menstrual cycle. After that, the serum was separated by centrifugation (3,000 for 10 minutes). The samples were then taken to the Department of Chemistry Biomass for

the purpose of measuring the various parameters (LH , FSH , TSH , Prolactin and testosterone) and compare them with healthy results.

Sample Collection

The study was carried in wasit - city, (120) patient in reproductive age were studied, (60) females were cases which were diagnosed polycystic ovary syndrome with different ages (18-40) years , (60) healthy females (control) different ages (18-40) years .These groups have been divided were the first group includes the age of (18 to 25) this (Group A) and in the second group the age was (26 to 40) this (Group B), measure the percentage of FSH , LH ,TSH , prolactin and testosterone in the blood , blood samples were collected and the history was taken from patients and their parents including: name, age and history of family cooperation with the control also divided in to two (Groups A) and (Group B) . The samples were collected in the on 3rd day of menses.

Statistical analysis

The statistical analysis of this study was used to analysis of variance in one direction (ANOVA) where it was used by the program Minitab , where it was adopted in this statistic the use of test (F) compare all the differences of variables that have been studied and also the results are significant if the value of (P) is less than 0.01.

Finding :

Table (1) the mean value of the parameter for females patients (group A)

Parameter	Control Age (17-25) n=30	Females patients (PCOS) Age(17-25) n=30	P- value
LH (IU/ml)	4.99	13.15	S
FSH(IU/ml)	4.63	7.85	S
Testosterone(ng/ml)	0.6	0.7	NO
Prolactin(ng/ml)	11.77	12.11	NO
TSH(IU/l)	2.7	5.8	S

S: refer to the parameter with the significant to the control

NS: refer to the parameter with the non-significant to the control

Table (2) the mean value of the parameter for females patients(group B)

Parameter	Control Age (26-40) n=30	Females patients (PCOS) Age(26-40) n=30	P- value
LH(IU/ml)	4.85	11.98	S
FSH(IU/ml)	4.66	7.80	S
Testosterone(ng/ml)	0.5.5	0.6	NO
Prolactin(ng/ml)	11.15	12.89	NO
TSH(IU/l)	2.4	5.5	S

S: refer to the parameter with the significant to the control

NS: refer to the parameter with the non-significant to the control

All of participants. were examined carefully at beginning and their demographic data such as age, weight, and history of medical state were written in information sheet. During day 2 - 4 of the cycle, hormone luteinizing (LH), hormone stimulating follicles (FSH), testosterone and serum hormonal level were performed, TSH ,and prolactin were measured. Show in the table (1) and table (2) show luteinizing hormone (LH) and follicle stimulating hormone (FSH) in day 2-4 of cycle where the LH levels shows highly significant Compared to good control, and show even show this levels FSH high significant in patient compared with healthy control , in this study shows women who have been diagnosed with polycystic ovaries high level LH is common and . There were also low FSH levels, statistically significant differences between normal and elevated LH and FSH ratios.

There were no differences between the table (1) and (2) in patients. Also, when compared to the normal weight with excess weight in patients, where it was observed to increase levels LH of the patients who have a high weight increase, although these differences are not important , Because it was observed to increase levels LH in patients with normal weight and overweight. The

levels of testosterone in patients with PCOS appear to be within normal range and this is did not help in the clinical diagnosis of polycystic ovaries, In addition, during the study it was noted in some patients increase the growth of coarse hair in areas of the body, such as the chin, chest, legs and abdomen,the difference between levels (group A) and (group B) is much lower. PRL ordinary proportions of most women with PCOS, but there is a slight increase in this hormone in women so there is no significant difference between patients and healthy levels of prolactin. The difference between levels (group A) and (group B) can also affect the reproductive cycle in PCOS. TSH levels appear to be significantly higher in patients with PCOS than in controlled healthy individuals and the difference between levels (group A) and levels (group B) is much lower. So symptoms of polycystic ovaries begin at an early age so it is necessary to diagnose the disease early and treat it.

Discussion

Anormal hormonal in women, the reproductive cycle is characterized by fluctuating gonadal hormonal levels. The negative control of FSH is critical for the development of a single mature oocyte which characterizes normal reproductive function in women. In this study, lower levels of FSH in PCOS indicate the persistence of this negative feedback to the extent that not one follicle can mature enough for ovulation. The cycles in PCOS should be taken into account because they are ovulatory and incorrect[10] . Although PCOS pathophysiology is unclear, it has been thought that a number of biochemical abnormalities are associated with this syndrome. so important the PCOS feature alters the dynamics of gonadotropin. In women with PCOS, several studies showed higher LH pulse and amplitude. Even if an increase in LH results in more androgens in the ovarian theca cells, the more immediate cause of anovulation may be insufficient follicle - stimulating hormone (FSH), Women in most PCOS[11] . And also the plasticity disturbance of the hormone releasing gonadotrophin (GnRH) as a result, LH release to FSH is relatively increased. An abnormal feedback mechanism by ovarian estrogen is blamed for playing a role in this discriminated increase in LH release[12] .

So the our study showed that Women with PCOS have higher levels of TSH and are also more likely to have subclinical hypothyroidism than controls without PCOS matched with age. According to the laboratory

guidelines of the National Academy of Clinical Biochemistry (NACB), 95% percent of regular thyroid volunteers with rigorous screening serum TSH values range from 0.4 to 2.5 IU / L4A 2011 compared to two groups. Women with hypothyroidism - one group with polycystic ovaries and the other group with normal ovaries - to a group with normal thyroid function, the researchers found hypothyroid women]13[. These findings suggest that the increase in TSH could be a risk factor for development and progression women's PCOS. based on these findings, to prevent metabolic disturbances induced by thyroid dysfunction, every woman diagnosed with PCOS should be examined for levels of TSH .Which can help prevent and treat endocrine at an early stage]14[. As mentioned earlier because of long term sequel of PCOS including infertility, endometrial hyperplasia, metabolic syndrome, and cardiovascular risk factor, early identification of at risk women would be very useful. Once the diagnosis of PCOS is made, additional evaluation is suggested including a cardio metabolic risk assessment, as well as screening for mood disorder and sleep apnea, screening for diabetes mellitus and for women pursuing fertility assessment of ovulatory status]15[.

Conclusion

The exact cause is unknown but genetic and environmental factors may contribute to its development.

Most patients women from group A and B in the age (18-25), (26-44) statistical significance no difference between groups.

Most of the patient women form group and B in the age (18-25), (26-44) statistical significance higher form hormonal (LH, FSH, TSH) when controlled healthy individuals .

Both groups have non-significance difference in hormone (Testosterone and Prolactin) when controlled healthy individuals .

Conflict of Interest: Non

Source of Funding : Self

Ethical Clearances : The consent of the patient has been taken in the hospital as well as approval from the university in order to initiate the work.

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Determination of Obstructive Sleep Apnea (OSA) among Type 2 Diabetes Mellitus at Diabetic and Endocrine Center in Al-Nasiriyah City.

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Abstract

The study aims to determine sleep apnea between type 2 diabetes and detect a relationship between sleep apnea and demographic characteristics (age, gender, duration of type 2 diabetes mellitus). A descriptive study design was conducted throughout the present study for period from 2 September 2017 to 1st March 2018. A purposive (non-probability) sample of 100 patients, the study was held at Diabetes and Endocrine Center in Al-Nasiriyah City. The data was collected using the created survey and the self-management bang process. The questionnaire was involved of three parts: patients' socio-demographic features (age, gender, occupation, once-a-month income and duration of type 2 diabetes mellitus). The additional part consists of Epworth Sleepiness Scale (ESS) of Obstructive Sleep Apnea (OSA). The validity of the tool content was strong-minded by using panel experts. The internal constancy of the instrument was strong-minded by the untried study and the significant correlation coefficient ($r = 0.870$) was determined. Data were investigated by applying descriptive and inductive statistical approaches using the Statistical Package for Social Sciences (IBMSPSS) version 20.0. The results revealed that the majority of the example fell in the age group 56 years. The results showed that the uppermost percentage of the sample was (unemployed) and was calculated (80.0%). The majority of the once-a-month income of the sample of the sample with insufficient and account (43.0%). The majority of the study sample has type 2 diabetes (6-10 years) and the account (32.0) of the study sample. (42.0) of patients with type 2 diabetes have obstructive sleep apnea. Strong correlation between duration of diabetes and Epworth Sleepiness (ESS) at P (value = 0.01).

Keywords: Obstructive Sleep Apnea (OSA), Patients, Epworth Sleepiness Scale (ESS).

Introduction

Type 2 diabetes is a clinical and chronic metabolic syndrome or disease that is rapidly growing around the world. Most important factors help in poor lifestyle and obesity (eg, more food intake and restriction of physical activity). Highly efficient procedure done to improve lifestyle and uses of modern multi anti diabetic drugs but the type 2 diabetes still account as economic and public health issue, Obstructive sleep apnea syndrome (OSAS) might activate hyperglycemia and insulin resistance (1). At the United States there are more than two third of population today are obese and overweight adults were treated from obstructive sleep apnea (OSA) which represent as treatable sleep problem. An

extensive amount of studies have demonstrated that OSA is related with insulin resistance, glucose bigotry and type 2 diabetes, paying little respect to obesity. Trial contemplate results on human and creature models that emulate OSA offers potential instruments of precisely how OSA may change glucose metabolism. Poor glucose control and worsening are strongly associated with type 2 diabetes patients which are suffering from undiagnosed OSA and account for 83% of all patients. Development of diabetes over a long period OSA remains unclear (2). Broad scale randomized-controlled studies of CPAP treatment of OSA with all around evaluation and assessments of insulin resistance and glucose intolerance are required. Obstructive sleep apnea represent as modifiable risk factors among prediabetes

and type 2 diabetes. The results of these studies confirm that (3) Late research displays the probability of a association between type 2 diabetes and obstructive sleep apnea (OSA), the most broadly apparent kind of sleep disorder. Global Diabetes Federation (IDF) declare that OSA and type 2 diabetes that should be increase attention about strong association between the two, that have positive impact on general health and individual daily life(4). Obesity represent a key factor of the consequence of OSA on type 2 diabetes mellitus, which adjusts glucose metabolism, enhance insulin resistance, and is accompanying with progress of type 2 diabetes.. Nevertheless, lengthy experience to recurrent hypoxia and other pathophysiological effects of OSA disturb glucose breakdown in directly way, and treatment of OSA able to increase glucose homeostasis. The most common chronic respiratory system disorder obstructive sleep apnea (OSA) is denoted (5). Pathophysiological complications of upper respiratory system collapse include intermittent hypoxia and sleep distribution, resulting in sympathetic stimulation, systemic inflammation, and oxidative stress. These sufferings affect and increased cardio metabolic morbidity and mortality detected in residents with OSA.

Method

Study design: A Descriptive was conducted though out (100) patients with type 2 diabetes mellitus

Study period: the study was conducted though out period from 2 September 2017 to 1st march 2018. at endocrine and diabetes center in Al- Nasiriyah city.

Sample of the study: randomize sampling of patient selection at endocrine and diabetes center in Al- Nasiriyah city. **Criteria:**

Age over than 30 years.

Patients accepted to cooperate in in study.

Patients male and female.

Type 2 diabetes patients.

Study Tools: In order to assess the presence of obstructive sleep apnea between type 2 diabetes, researchers use the questionnaire:

Part1: Patients socio demographic characteristics includes (age, gender, occupation, educational level, address, monthly income, how long the duration of

having diabetes mellitus, have another disease plus to diabetes mellitus).

Part 2: Questionnaire consist of seven domains: Epworth Sleepiness Scale (ESS) will be utilized to classify obstructive sleep apnea between type 2 diabetes. The Epworth Sleepiness Scale is broadly used in the field of sleep medicine as an inventory scale of patient sleep. The test is a rundown of eight cases in which measure the inclination to feel tired on a size of zero, and there is no possibility to stop, to 3, a high shot of stopping. When complete the process of testing, include the estimations of your reactions. Your score relies upon a scale from 0 to 24. The measure gauges whether you are encountering have extensive sleepiness that may require therapeutic consideration.

Scoring and Rating:- The items was rated and scored according to the following designs:

- Do not have a accidental to sleep = 0
- A slight occasion of drowsiness = 1
- Suitable reasonable dozing = 2
- Tall chance of sleep o = 3

0-7: It is unlikely to be abnormally sleepy.

8-9: Patients have an average of sleepiness during the day.

10-15: The patient may be overly sleepy as the case may be. You may want to reflect seeking medical consideration.

16-24: Patients are extremely sleepy and should consider looking for medical care.

Data collection:- The data when collected with built questionnaire though an request direct interviewing and indirect answers as nasty of data collection.

Statistical analysis:- Analyzing data by using IBM. SPSS (Statistical Package for Social Sciences) version to data was presented as number and percent data analyzed though an application of frequency and Person correlation test to determine the association.

Findings

Table 1: Distribution of socio-demographic data of patients with Type 2 Diabetes Mellitus

Basic Information	Groups	Frequency	Percent
Age of patients	25-35 years	14	14.0
	36-45 years	13	13.0
	46-55 years	26	26.0
	56 years and more	47	47.0
	25-35 years	14	14.0
	Total	100	100.0
Gender	Male	54	54.0
	Female	46	46.0
	Total	100	100.0
Occupation	Employee	18	18.0
	Unemployed	80	80.0
	Student	2	2.0
	Total	100	100.0
Residency	City	74	74.0
	Rural	26	26.0
	Total	100	100.0
Monthly Income	Sufficient	29	29.0
	Insufficient	43	43.0
	Barely insufficient	28	28.0
	Total	100	100.0

Table (1) It is noted that (47.0%) of patients in the age group more than 56 years. With respect to gender, the large number of the sample is male and female (54.0%). With regard to the state of job, the results designated that the highest percentage of the sample of the study are (unemployed) and are calculated (80.0%). With regard to the residency of patients, the majority of the study sample lives in the city by (74.0). The majority of the once-a-month income of the study sample is inadequate and is account for (43.0%).

Table 2: Distribution of Duration of Type 2 Diabetes Mellitus among (100) Patients

	Frequency	Percent	
Duration of Type 2 Diabetes Mellitus	1-5 years	40	40.0
	6-10 years	32	32.0
	11-15 years	17	17.0
	16-20 years	9	9.0
	Over than 21 years	2	2.0
	Total	100	100.0

Cont... Table 2: Distribution of Duration of Type 2 Diabetes Mellitus among (100) Patients

	Frequency	Percent	
Epworth Sleepiness Scale (ESS)	0-7: It is unlikely to be abnormally sleepy.	41	41.0
	8-9: Patients have an average of sleepiness during the day.	17	17.0
	10-15: The patient may be overly sleepy as the case may be. You may want to reflect observing for medical attention.	31	31.0
	Patients are extremely drowsy and must consider seeking medicinal attention.	11	11.0
	Total	100	100.0

Table (2) show the majority of study sample have type 2 diabetes since (6-10 years) and account (32.0) of study sample.

Table (3): Distribution of Epworth Sleepiness Scale (ESS) among Type 2 Diabetes Mellitus:

Table (4): Correlation between (Age of patients, Gender, Duration of Type 2 Diabetes Mellitus and Epworth Sleepiness Scale (ESS))

Age of patients * Epworth Sleepiness Scale	Pearson Correlation	-.025-
	Sig. (2-tailed)	.805
	N	100
Gender of patients* Epworth Sleepiness Scale	Pearson Correlation	.059
	Sig. (2-tailed)	.557
	N	100
Duration of diabetes mellitus* Epworth Sleepiness Scale	Pearson Correlation	.042**
	Sig. (2-tailed)	.676
	N	100

Table (4) show the strong statically correlation between Duration of diabetes Mellitus and Epworth Sleepiness Scale (ESS) at (P value=0.01).

Discussion

Part-I: Distribution of 100 patients with type 2 diabetes according to demographic characteristics:

The results of the study had shown that indicated that (47.0 %) of the patients within age group additional than 56 years this results reinforced by (6) With regard to gender, the largest number of the sample is male and female (54.0%), this results are supported by study done

by (7). Related to occupation state, the results designated that the uppermost percentage of the sample of the study are (unemployed), it is account for (80.0%). With regard to the residency of patients, the majority of the study sample lives in the city and represent for (74.0). The majority of the monthly income of the study sample is insufficient and is calculated (43.0%).

Part-II-: Distribution of Duration of Type 2 Diabetes Mellitus among (100) Patients:

The majority of study sample have type 2 diabetes since (6-10 years) and account (32.0) of study sample, that was agree with study complete by (8).

Part-III:- Correlation between (Age, Gender, Duration of Type 2 Diabetes Mellitus and Epworth Sleepiness Scale (ESS)

The results of study sample show the strong statically correlation between Duration of diabetes mellitus and Epworth Sleepiness Scale (ESS) at (P value=0.01) . These results are reinforced by (9).

Conclusions:The highest percentage of the study sample was male, more than female, illiterate, reading and writing, elementary school and married. They were unemployed and did not have enough monthly income. Determination of obstructive sleep apnea between type 2 diabetes shows more than a third of the study sample have obstructive apnea. These patients were hinted and not discovered.

Ethical Clearance: Permission from diabetic and endocrine center, in Al-Nasyriha directorate was attained before starting the study. All the participants gave verbal consent with confidentiality of participants' identification

Conflict of Interest: Nil

Source of Funding: Nil

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Obstetrical and Medical History of Pregnant Women with Toxoplasmosis in Al-Najaf Province, Case-Control Study

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Abstract

Background : Bad obstetric history implies previous unfavorable foetal results such as consecutive spontaneous abortions for two or more, early neonatal death, have intrauterine foetal death, stillbirth, intrauterine growth retardation, and congenital deformities. This study aims to assessment the obstetrical and medical history of pregnant women with toxoplasmosis in Al-Najaf province. **Materials and Methods:** A case control study was conducted in Al Zahra Teaching Hospital in Al-Najaf province. The cases in this study were the pregnant women with toxoplasmosis and the pregnant women without toxoplasmosis as (control). A number of 300 respondents were chosen using simple random sampling method. **Findings:** Most of the participants were in the age group (21-25) years for both groups. Most of participants in both groups have family income within range 401.000-800.000 IQD. Most of participants in both groups have primary level of education. Regarding to the occupations, most of the participants in both groups were housewives. Most of the participants in both groups were living in urban area without any statistical difference between groups. In addition, most of the cases have miscarriages (86%) following by stillbirth (18.7%), deformities (10.7%) and premature delivers (8%). While, the control group 11.3% had miscarriages 5.3% stillbirth and 45% premature delivers. It also shows that there are statistical significant differences between groups for items miscarriages stillbirth, deformities antiphospholipide syndrome and diabetes. **Conclusion:** The odds ratio indicated that there was almost 7 items of 9 of obstetrics and medical history of cases were risk factor. While, for control group was protective factors.

Keyword: *Obstetrical, medical history, pregnant women, toxoplasmosis.*

Introduction

Bad obstetric history (BOH) implies previous unfavorable foetal results such as consecutive spontaneous abortions for two or more, early neonatal death, have intrauterine foetal death, stillbirth, intrauterine growth retardation, and congenital deformities (1). The reasons of BOH might be related to hormonal effect, abnormal maternal immune response, genetic effect, and infection during pregnancy (2,3). The prenatal and perinatal infections are a medical acronym for a set of perinatal infections, such as infections that are passed to the fetus from the infected mother (4). Some pregnant women had some complicated such as pre-eclampsia or spontaneous preterm birth that might be increased risk of recurrence in the other pregnancies (5).

The other group of women with history of preterm premature rupture of membranes in their first pregnancy are at an increased risk for pregnancy-induced operative delivery, hypertension and pre-eclampsia (6). These outcomes lend further confirmation to the suggestion that all of these bad prognosis represent indicator to be more attention about these women (5). Morbidity during maternal period includes psychological and physical conditions that result from or are aggravated by pregnancy and have unfavorable effect on a woman's health. The most dangerous complications that been occur during pregnancy, generally can be called as severe maternal morbidity (7). This study aims to assessment the obstetrical and medical history of pregnant women who were infected with toxoplasmosis in Al-Najaf province.

Material and Method

Study Location

This study was conducted in Al-Najaf province, one of the eighteen provinces in Iraq. Al-Najaf Province consists of six districts (8). In 2006 Al Najaf had a population of 1,042,900, which represent 3.7% of total population in Iraq(9). The respondents in this study, were taken from the Obstetrics and Gynecology unit in Al Zahra Teaching Hospital in Al-Najaf city.

Study design and Sample

A case control study was conducted in Al Zahra Teaching Hospital in Al-Najaf province. The respondents in this study were the pregnant women with toxoplasmosis (cases) and the pregnant women without toxoplasmosis as (control).The sampling population consist of 860 pregnant women with toxoplasmosis that fulfilled the inclusion and exclusion criteria. A number of 300 respondents were chosen using simple random sampling method, 150 for cases and 150 for control. The p in the calculation of the sample size is taken from the previous study (10), with confidence interval $p = 0.05$ and power of study 1.645.

Inclusion and Exclusion Criteria

Inclusion criteria

Inclusion criteria is the pregnant women who were attended to the gynecological clinic at Al Zahra Teaching Hospital in Al-Najaf province during the peroid of this study and they were infected with toxoplasmosis.

Exclusion criteria

Exclusion is the pregnant women who were attended to the gynecological clinic at Al Zahra Teaching Hospital in Al-Najaf province during the period of this study and they werent infected with toxoplasmosis. And they werent from Al-Najaf province.

Data collection

Data was collected from 300 eligible respondents, who had given their consent to participate in this study. A self-administrated questionnaire in the Arabic Language was used to collect information. The whole questionnaire has three sections, section one was about sociodemographic characteristics which included age, education level, family income, living area and occupation. Section two was about obstetrical history and section three was about the past medical history.

Data Analysis

Statistical Package for the Social Sciences (SPSS) version 23 was used to analyze the data(11). For descriptive and inferential statistical, the frequencies, percentages, mean and standard deviation, were calculated for selected numerical and categorical variables. T-test was used to test the difference between the numerical data and Mann-Whitney to test the difference between the categorical data. The dependent variable is the pregnant woman who has positive laboratory evidence of toxoplasmosis infection, whereas the independent variables are the socio-demographic characteristics, Obstetrical and medical history.

Findings

Table (1) Distribution of sociodemographic characteristics of respondents (Case and Control)

P value a,b	No. and percentages	Age group Years	
	Control (N=150)	Cases (N=150)	
			Rang (15-≥50)
	29.4 ± 7.9	26.2 ± 6.9	Mean ± SD
0.001a	21(14%)	33 (22%)	15-20
	35(23.3%)	51(34%)	21-25
	35(23.3%)	25(16.7%)	26-30
	20(13.3%)	25(16.7%)	31-35
	24(16%)	9(6%)	36-40
	12(8%)	6(4%)	41-45
	3(2%)	1(0.7%)	46-50

Cont... Table (1) Distribution of sociodemographic characteristics of respondents (Case and Control)

			Family income*(IQD)
0.001a	729913.3 ± 270601.4	523493.3 ± 279475.1	Mean ± SD
	15(10)	66(44)	100-400
	84(56)	62(41.3)	401-800
	42(28)	16(10.7)	801-1200
	9(6)	6(4)	≥1201
			Educational Level
0.782b	39(26)	27(18)	Never been to school
	55(36.7)	66(44)	Primary level
	21(14)	34(22.7)	Secondary level
	6(4)	9(6)	Tertiary level
	13(8.7)	5(3.3)	Diploma
	15(10)	8(5.3)	Bachelor
	1(0.7)	1(0.7)	Master
0.009b			**Occupation
	128(85.3)	142(94.7)	Not working\Housewife
	6(4)	1(0.7)	LSOM
	8(5.3)	2(1.3)	professionals
	3(2)	2(1.3)	Technicians And associate Professionals
	1(0.7)	1(0.7)	Clerks
	3(2)	1(0.7)	Service Workers, Shop, Market Workers
	1(0.7)	1(0.7)	Skilled agriculture and fishery workers
			Geographical area
0.700b	115(76.7)	106(70.7)	Urban
	35(23.3)	44(29.3)	Rural

a-t-test significant value at $p < 0.05$

bMann-whitney test at $p < 0.05$

* Iraqi dinar (thousand) (1000 IQD = 0.844 USD (2018))

**Occupation- according to the I.L.O

Table (1) shows that the age range of respondents was (15-<50) years. Mean ± SD (26.2±6.9) for cases and (29.4±7.9) for control and there is significant difference between them (0.001). Most of respondents were in the age group (21-25) years for both groups. As for family income, there is significant difference between groups (0.001). According to the education level, most of respondents had primary level of education. As for occupation, most of respondents for both groups were housewives. Also, found that most of respondents were from urban areas.

Table (2) Obstetrical and medical history of respondents (Case-Control)

Obstetrical Items	No. and percentages		P-value Sig. *
	Cases	Control	
Miscarriages	129(86%)	17 (11.3%)	0.001
Stillbirth	28(18.7)%	8 (5.3%)	0.001
Deformities	16(10.7)%	4 (2.7%)	0.006
Premature Deliveries	12(8%)	6 (4%)	0.145
Unexpected neonatal death	4 (2.7%)	1 (0.7%)	0.177
Antiphospholipids Syndrome	2 (1.3%)	3 (2%)	0.001
Blood transfusion	21 (14%)	34 (22.7%)	0.053
Diabetes	11(7.3%)	3(2%)	0.029
Hypertension	6(4%)	3(2%)	0.311

*mann-whitney test

The results of this table shows that 7 obstetrical and medical items of 9 have significant difference between groups.

Table (3) Crude odds ratios (95% confidence intervals) and logistic regression model of obstetrical and medical history for both groups (case and control)

Obstetrical Items	Odds ratio (95% CI)	
	Cases	Control
Miscarriages	6.479 (4.335 to 9.684)	0.135 (0.086 to 0.212)
Stillbirth	1.683 (1.354 to 2.093)	0.413(0.222 to 0.769)
Deformities	1.672 (1.301 to 2.148)	0.384 (0.159 to 0.928)
Premature Deliveries	1.362 (0.962 to 1.929)	0.653 (0.336 to 1.267)
Unexpected neonatal death	1.616 (1.027 to 2.543)	0.396 (0.068 to 2.294)
Antiphospholipids Syndrome	0.797 (0.271 to 2.347)	1.204 (0.583 to 2.486)
Blood transfusion	0.725 (0.508 to 1.036)	1.306 (1.021 to 1.670)
Diabetes	1.617 (1.200 to 2.179)	0.417 (0.152 to 1.144)
Hypertension	1.347 (0.837 to 2.169)	0.660 (0.260 to 1.674)

Table (3) reveals that the respondents in the cases group (toxoplasmosis) have odds ratio toward risk factors. On other hand, the respondents in control group have odds ratio toward normal level.

Table (4) Correlation between obstetrical history and socio-demographical characteristics of respondents for both groups

Obstetrical history								
	Age	Education	Occupation	Geo				
	Case	Con.	Case	Con.	Case	Con.	Case	Con.
Miscarriages	0.074	0.229	0.043*	0.019*	0.115	0.638	0.971	0.998
Stillbirth	0.529	0.131	0.223	0.787	0.073	0.000**	0.911	0.827
Deformities	0.424	0.464	0.656	0.422	0.997	0.500	0.232	0.782
Premature Deliveries	0.926	0.884	0.116	0.984	0.937	0.406	0.657	0.944
Unexpected Death	0.926	0.354	0.021*	0.152	0.486	0.739	0.163	0.527
Antiphospholipids	0.103	0.001**	0.213	0.703	0.815	0.559	0.521	0.669

*Correlation is significant at level 0.05

** At level 0.01

This table shows that there are significant correlation at level 0.01 between age and antiphospholipids for control group. As for education, there is correlation at level 0.05 between miscarriages and education for both groups, and between unexpected neonatal death and education for case group.

Table (5) Correlation between Obstetrical history and medical history for both groups

Obstetrical history	Blood Trans	Hypertension	Diabetes			
	Case	Con.	Case	Con.	Case	Con.
Miscarriages	0.527	0.353	0.166	0.227	0.680	0.227
Stillbirth	0.212	0.729	0.350	0.680	0.966	0.680
Deformities	0.182	0.000**	0.000**	0.000**	0.000**	0.000**
Premature Deliveries	0.558	0.309	0.428	0.723	0.891	0.723
Unexpected Death	0.524	0.683	0.681	0.887	0.572	0.887
Antiphospholipids	0.019*	0.797	0.021*	0.187	0.001**	0.187

*Correlation is significant at level 0.05

** At level 0.01

The results of table (5) shows that there are significant correlation at level 0.01 between blood transfer, hypertension and diabetes with deformities for both groups and with antiphospholipids for cases group at level 0.05.

Table (6) Correlation between Obstetrical history items for both groups

Obstetrical history	Obstetrical history											
	Miscarriages	Stillbirth	Deformities	Premature Deliveries	Unexpected Death	Antiphospholipids						
	Case	Con.	Case	Con.	Case	Con.	Case	Con.	Case	Con.	Case	Con.
Miscarriages	1	1										
Stillbirth	0.517	0.302	1	1								
Deformities	0.565	0.013*	0.993	0.633	1	1						
Premature Deliveries	0.783	0.084	0.854	0.556	0.787	0.681	1	1				
Unexpected Death	0.524	0.722	0.335	0.813	0.487	0.869	0.000**	0.839	1	1		
Antiphospholipids	0.729	0.051	0.883	0.146	0.163	0.312	0.633	0.542	0.048*	0.791	1	1

*.Correlation is significant at level 0.05

**At level 0.01 level

The results of table (6) shows that there are significant correlation at level 0.05 between miscarriages and deformities for control group. In addition, there is significant correlation at level 0.01 between premature deliveries and unexpected neonatal death for case group. Also, there is significant correlation at level 0.05 between unexpected neonatal death and antiphospholipids for case group.

Discussion

This study was found that most of the participants were in the age group (21-25) (34% and 23.3%) for case and control respectively with statistical significant difference between groups ($p = 0.001$) (Table 1). This results almost were near to the result found in Kirkuk in the north of Iraq in 2013, they found that most of the participants were in age less than 30 years (12). It also reveals that the age range was (15- \geq 50) years with mean \pm SD were 26.2 ± 6.9 for cases and 29.4 ± 7.9 for control group, it's also shows that there is statistical

significant difference between groups ($p = 0.001$). Lt and Maj Found that there was notstatistical significant difference between groups ($p = 0.181$) between groups who had bad obstetrical history (13). That means there is difference between communities however, there was not difference between communities within the country.

According to the family income, the result of this table shows that the most participants in both groups have family income within range 401.000-800.000 IQD and there is statistical significant difference between groups ($p = 0.001$)(Table 1). At the same place in one study was conducted in 2016, the researchers were found that the mean average of monthly family income was IRD 529,000 (USD241) for the Experiment group and IRD 719,000 (USD694) for the Controlgroup (8). This is indicator to poor the situation of living style for those people.

For the educational level, it shows that most of the cases have secondary level of education (22.7%). While, the control group most of them have primary

level of education (36.7%) and there was not statistical difference between groups ($p = 0.078$)(Table 1). This results were agree with the result found in Kirkuk in the north of Iraq in 2013, they found that most of participants in both groups had low level of education (12). It also agree with result of another study was carried out at the same place in 2016, the found that both groups had low level of education (45.3% for Experiment and 37.1% for the Control group)(8).

Regarding to the occupations, most of the participants in both groups were housewives (94.7% for cases and 85.3% for control). It also shows that there was statistical significant difference between groups ($p = 0.009$)(Table 1). Aljumaili and Alsamarai at Kirkuk in 2013 found that same result, they found most of the participants were housewives in addition to results of Atheer and Titi in 2016 (12,8). This result found that same finding that been founded by Aljumaili and Alsamarai, 2013; Ibadi and Hamedon, 2016 regarding to the geographical distribution of participants, most of the participants in both groups were living in urban area without any statistical difference between groups.

According to the obstetrical and past medical history, the results of this study show that significant difference between two groups towards the cases group. It's also found that the obstetrical history of cases group was more than control group. However, the past medical history for case group was more than control (Table 2).

One study was conducted in Khammam, Andhra Pradesh, India in 2012 was agree with our study, the researcher were found that the abortion cases was the commonest form of pregnancy wastage (51.92%), followed by stillbirths (36.53%) and premature deliveries (7.69%). This result was significantly higher in the study group than that in the control group, and the seropositivity with toxoplasmosis played an important role in determining the foetal outcome (14). Another study was conducted in Egypton 2015 on pregnant women with toxoplasmosis. This study was found that the seroprevalence of pregnant women with toxoplasmosis was significantly different from that of normal pregnancy group ($P < 0.05$). It also found that the infected women in relation to bad obstetric history showed that abortion was the commonest pattern of the pregnancy wastage (56.5%), this result is also agree with our results (15).

The results of this study show that the participants who have toxoplasmosis with obstetrical and medical history have risk factor to get miscarriages Odds 6.479 (4.335 to 9.684) however, this situation was different for control Odds 0.135 (0.086 to 0.212). For stillbirth, deformities, premature delivers and unexpected neonatal death were also have risk factor for these items 1.683 (1.354 to 2.093), 1.672 (1.301 to 2.148), 1.362 (0.962 to 1.929) and 1.616 (1.027 to 2.543) respectively. However, for the control group were significant protective factors 0.413 (0.222 to 0.769), 0.384 (0.159 to 0.928), 0.653 (0.336 to 1.267) and 0.396 (0.068 to 2.294) respectively (Table 2,3). The odds ratio for cases was risk factor for almost 7 items of 9 of obstetrical and medical history. However, for control group was protective factors. The study was conducted in Egypt on 2015 on pregnant women with toxoplasmosis was found that there was significantly different between odds ratio values between groups ($P < 0.05$) this result is also agree with our results (15).

Conclusion

Most of the participants were in the age group (21-25) years, have primary level of education and were housewives.

Both groups have family income within range 401.000-800.000 IQD.

Most of the cases have miscarriages following by stillbirth, deformities and premature delivers. While, control group have miscarriages following by stillbirth and premature delivers.

The odds ratio for cases was risk factor for almost 7 items of 9 of obstetrical and medical history. However, for control group was protective factors.

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Ethical Clearance: Written consent was taken from all respondents in this study, official consent was also taken from Al-Najaf Health Directorate\ Center of Training and Development of Staffs (ref: No.136830), this work was done according to the ethical committee considerations of Al-Furat Al-Awsat Technical

University and Al-Najaf Health Directorate.

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Study The Phytochemical Analysis and Modulatory Effects of Hydroalcoholic Extract of Morus Plant Leaves on Mental Depressive Disorder

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Abstract

Objectives: To evaluate the antidepressant activity of hydroalcoholic extract of Morus alba leaves in mice after acute 7 days and 14 days of treatments by using two models of depression tail-suspension test (TST) and forced swim test (FST). **Duration:** Three months (September 2018-December 2018). **Design:** Observational study by using digital camera. **Findings:** The findings showed that the hydroalcoholic extract of Morus alba leaves at the doses of 200 and 400 mg/kg significantly ($P > 0.05$) reduced the immobility times in both the tests (TS & FS) as compared to control in both acute and after 14 days of treatments. The phytochemical analysis of hydroalcoholic extract of Morus alba leaves revealed the presence of alkaloids, phenols, flavonoids, Tannins, Saponins, Steroids Glycosides and Coumarins. **Conclusion:** It can be concluded that Morus alba hydroalcoholic leaves extract have antidepressant effect in both acute and after 14 days of treatment and there were dose dependent in increasing these effects.

Keywords: Morus alba , antidepressant, Mice

Introduction

Major depression is a mental disorder in psychiatric practice where that patient presents with one or two major symptoms sadness and anhedonia 1. Various drugs are available for treatment of depression but approximately two-third of depressed patients respond to the available treatments², beside these drugs have unusual side effects and the chronic use of these pharmacological agents that chemically synthesized is associated with serious adverse effects, therefore there is a need of alternative safe effective agents to treat and counteract these adverse effects³. Now there is moving towards use of herbal compound as a choice of therapy instead of using the chemical agents⁴. These drugs of plant sources have low side effects beside they are good source as antioxidants for attenuating diseases. Morus alba is one of these plants that has various pharmacological properties. The leaves and roots have been used as cathartic, analgesic, diuretic, antitussive and hypotensive⁵. Decrease the levels of monoamine neuromediators and oxidative stress are important factors in the pathogenesis of neurological

and neuropsychiatric disorders⁶. The leaves of Morus alba are known to contain flavonoids like quercetin which render the antidepressant property⁷ and quercetin has also been shown to inhibit Catechol-O-Methyl Transferase (COMT) and monoamine oxidase (MAO) in brain⁸. Certain resistant cases of depression which do not respond to modern remedies may respond when treated with herbal preparations beside the adverse effects of antidepressants can be overcome if alternate medicines are used.

Materials and Method

The study was carried out in the Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Baghdad. Adult male albino mice (Swiss strain) weighing 20-30 gm were obtained from the animal house of Iraqi Center of Cancer Research, Baghdad. The mice were included in the study housed at the animal house of the College of Veterinary Medicine, Baghdad under suitable conditions with a 12 hours light and dark cycle. They were fed with commercial pelleted chow and water ad libitum. They

were allowed to acclimatize for these conditions for one week before beginning of the experiments .

Hydroalcoholic leaves extract:

Freshly leaves of *Morus alba* L. collected from local trees of Baghdad ,Iraq during September and October 2017 .The leaves were dried about (1-7) days in room temperature shade . The dried leaves were pounded to a fine powder by using an electrical grinder and kept in cleaned container .The plant leaves were classified previously in the Ministry of Agriculture , State Board for Seeds Testing and Certification in Abu-Gharib , Baghdad ,Iraq. The *Morus alba* leaves powder was extracted according to 9.

Phytochemical analysis of Hydroalcoholic leaves extract:

The phytochemical analysis of *Morus* leaves extract was done according to the method that described by 10.

Drugs and Solutions :

Imipramine drug (NOVARTIS, India) was used .The standard solution of imipramine was prepared by dissolving 25 mg tablet in 25 ml distilled water to get a concentration of 1 mg /ml and given at a dose 10 mg/kg orally 11.Methanol (Sigma chemical co. St Louis ,USA), Distilled water , *Morus alba* leaves extract was given at doses of 200 mg/kg and 400 mg/kg 12. All the drugs were given to mice orally as a single dose once daily at a volume of 0.1 ml /10 gm B.W.

Experimental design:

The experiments were carried out during the light phase of the cycle and each animal was used only once .To evaluate the antidepressant activity of *Morus alba* leaves extract in acute study (7days) of treatment animals were divided into four groups ,five mice each .Group I (-ve control) was received distilled water. Group II (+ve control) was received standard antidepressant drug imipramine at a dose of 10 mg /kg. Groups III and IV were received leaves extract at doses of 200 and 400 mg/kg respectively .To evaluate the antidepressant activity of *Morus alba* leaves extract after 14 days of treatment , another different four groups of mice were used , five mice each which arranged as the same as that in the acute study . On the 7th and 14th days of treatment the mice in all groups after 30 minutes of drugs administration were tested in TST and FST.

Tail suspension test:

The method was described by 13& 14.The test is one of the most widely used model for evaluating the antidepressant activity in mice which suspended by their tail will develop an immobile posture . Each mouse was given 1 trail that last 6 min .The immobility time was recorded as the main parameter.

Forced swim test:

The forced swim test was developed by 15. Animals were forced to swim in a Plexiglas cylinder which filled with tap water at 22-23 C° to about 10 cm height . After an initial 2 min . period each mouse assumed and typical immobile posture . A mouse was considered to be immobile when it remained floating in the water.The total immobility time was recorded during the next 4 min . of the total test duration of 6 min . Each mouse was used only once.

Statistical method:

Data are presented as mean \pm S.E. which were analyzed by using completely randomized design in factorial experimental (One-way) ANOVA SPSS packag (2008). A probability of ($P > 0.05$) was considered as significant differences 16.

Findings

The percentage of extract that obtained was 27% .The phytochemical analysis of the extract revealed the presence of Alkaloids , Phenols , Flavonoids , Tannins , Saponins , Steroids ,Terpenoids , Glycosides and Coumarins (table 1).

Table 1: Phytochemical analysis of the extract

No	Phytochemical constituents	Identification
1	Alkaloids	+++
2	Phenol	+++
3	Flavonids	+++
4	Tannins	+++
5	Saponins	++
6	Steriods	+
7	Terpenoids	+
8	Glycosides	+
9	Coumarins	+

To evaluate the antidepressant activity of the extract, two models were used, tail suspension and forced swim tests in mice that were suspended by their tails or forced to swim in water from which they cannot escape and they will show a characteristic immobile postures which serve as screening models for antidepressants 17. The results presented in table (2 and 3) which showed that in acute study the antidepressant activity of Morus alba leaves extract were compared with standard antidepressant drug imipramine and revealed a significant ($P > 0.05$) decreased in immobility times in both TS and FS tests at both doses of 200 and 400 mg/kg of Morus alba leaves extract respectively which compared to (-ve) control.

Table 2 : Effects of treatment with extract on immobility time (TST) after 7 days of treatment:

Groups	Immobility time (Second) Mean± S.E.
I : Distilled water orally (-ve control)	170.22 ± 7.96 C
II: 10 mg /kg of imipramine (+ ve Control)	90.20 ±1.15A
III : 200 mg/kg of extract	130.52 ±3.40B
IV: 400 mg /kg of extract	96.15 ±1.66A

Table 3: Effects of treatment with extract on immobility time (FST) after 7days of treatment :

Groups	Immobility time (Second) Mean± S.E.
I : Distilled water (-ve control)	100.33 ±1.72C
II: 10 mg /kg of imipramine (+ ve Control)	42.20 ±0.20A
III : 200 mg/kg of extract	80.40 ±1.42 B
IV: 400 mg /kg of extract	44.20 ±1.16A

The findings after 14 days of treatment as shown in tables (4 and 5) showed the leaves extract at a dose 400 mg/kg significantly ($p > 0.05$) decreased the immobility times (seconds) in both tests as shown in tables 4 and 5 as compared to (-ve) control group.

Table 4 : Effects of treatment with extract on immobility time (TST) after 14 days of treatment:

Groups	Immobility time (S e c o n d) Mean± S.E.
I : Distilled water (-ve control)	170.00 ± 8.67 C
II: 10 mg /kg of imipramine (+ ve Control)	55.21 ±3.00A
III : 200 mg/kg of extract	100.31 ± 5.30 B
IV: 400 mg /kg of extract	65.20 ± 3.42A

Table 5 : Effects of treatment with extract on immobility time (FST) after 14 days of treatment:

Groups	Immobility time (Second) Mean± S.E.
I : Distilled water (-ve control)	92.20 ± 2.00 C
II: 10 mg /kg of imipramine (+ ve Control)	33.22 ±0.88A
III : 200 mg/kg of extract	60.00 ± 2.30 B
IV: 400 mg /kg of extract	35.44 ±1.63A

Discussions

Depression is a dominant type of neuropsychiatric disorders 18. The disease is going to be the second cause of impairment by 2020 and the first by 2040 according to the predicted by WHO 19 .On the basis of the clinical observation of depressive disorders and stressful life the TST and FST are the most widely used animal models which are specific to all major classes of antidepressants 20. In both tests the immobility reflect states of behavioral despair in mice which produced a condition that similar to human depression 21. TST has higher pharmacological sensitivity than FST 22 & 23 . In the present study the results revealed that the administration of hydroalcoholic extract of Morus alba leaves at doses 200 and 400 mg/kg was effective as

antidepressant and these effects increase with increasing the dosage of extract which increase more after 14 days of treatment than in acute state and there were dose dependent decreased in immobility times with extract .Our work is an agreement with 7 & 24. However 25 reported that the odor from the green leaves was shown to have serotonergic system mediated antidepressant activity in FST in mice and it has been reported to inhibit COMT and MAO enzymes in the brain.The results of phytochemical analysis revealed that extract contained alkaloids , flavonoids , tannins ,phenolic compounds, saponins terpenoids and glycosides which have antidepressant activity 26 &27

The exact mechanism of antidepressant activity of leaves extract is clearly not known but the experimental studies showed that leaves contain flavonoids like quercetin which is 3,5,7,3,4-pentahydroxy flavone which is potent bioflavonoid present in various vegetables and fruits that inhibit COMT and MAO enzymes in brain which increases the concentrations of catecholamines in the synaptosomes and attenuates depression. 8

Oxidative stress is one of the mechanisms that involved in neuropsychiatric problems . Nitric oxide (NO) is one of free radicals that have role in stress and depression either by modulating the release of other neuromediators acting as a cellular communicator in plasticity and development or acting as a vasodilator in the regulation of blood flow 28.The changes of the neuronal nitric oxide synthase activity and the antioxidant systems in patients with affective disorders have been demonstrated29.Quercetin and other flavonoids are present in hypericum perforatum that inhibit NOS in blood and cerebral homogenate which involved in antidepressant effect of plant 30. Morus alba leaves extract contain flavonoids that possess significant radical scavenging property and decrease expression of nitric oxide synthase in the hypothalamus of diabetic rats may contribute to its antidepressant activity 31 & 32. It has also shown the antioxidative and antithrombotic protective effects in LDL-receptor –deficient mice 33.Thus it may be possible that the extract in addition to inhibit COMT and MAO in brain , its antioxidant activity may responsible as antidepressant.

Conclusion

Leaves extract has significant antidepressant activity in both acute and after 14 days of treatment

which compared to standard antidepressant drug imipramine and there were dose dependent in increasing these effects. Further studies are required in lab animals and in long periods of treatments to determine the exact mechanism of its antidepressant to potentiate our results and further studies in human may be needed to confirm the results of animal studies .

Source of Funding: Self

Conflict of Interest: Non

Ethical Clearance: The study was performed under the guide lines supervision of Ethical Committee for lab . animals work in the College of Veterinary Medicine , University of Baghdad .

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Study of Relationship between Asthma and Obesity in Al Zahraa center for asthma Allergy in Baghdad City

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Abstract

Recently, the occurrence of asthma and obesity has increased. In this study, the main aim is to examine if asthma and obesity are related. Samples which collected from patients who attended Chest Diseases Department, Al Zahraa Center for Asthma Allergy between August 2017 and April 2018. A total of 100 individuals aged 6–73 with a mean age of met the entry criteria. In this study the most common age of cases and control was <20 years which represents 68% and 52% of study population respectively. Mean of body mass index was observed in asthmatic patients (23.85 ± 6.248) when compared to non-asthmatic subjects (25.68 ± 5.744), with no association (p -value = 0.091). A significant association was found between asthmatic patients when they were compared with that of control groups ($p = 0.000$) for all Spirometrical results. Obesity show not significant decrease in asthmatic patients as compared to healthy control group. All spirometrical result show highly significant decrease in asthmatic patients as compared to healthy control group.

Key words : Asthma, obesity, Spirometry, expiratory volume, vital capacity

Introduction

The occurrence of asthma and obesity was increased recently in spite of the development of public health care in United States (1). A correlation between obesity and asthma was suggested because of their prevalence (2). Age-adjusted occurrence data taken from the National Health and Nutrition Examination Survey indicated that 65 of adults in US are experiencing obesity, with a rise of 10 percent from 1988–1994 to 1999–2000 (3,4). Even though that asthma is not as much widespread as obesity, it have an effect on about 7 percent of adults in United States (5). A lot of cross-sectional epidemiologic researches indicated a modest relation between prevalent asthma and obesity (6–9). Through the use of varying obesity definitions, the relative risk regarding asthma in obesity range from 1.0 (no influence) to 3.0 (6, 8, 9). Nevertheless, although that these studies regularly control for confounding through diet, age, social and economic status, and levels of activity, causation in the relation between asthma and obesity could not be concluded decisively from only the cross-sectional data. Even though that a lot of researchers have explained

these data for suggesting that the risk of asthma increases by obesity, one might not exclude that asthma could influence obesity, possibly because of inactivity or systemic corticosteroid side effects (10). The main aim of this study has been examining the correlation between the severity of asthma and BMI in Al Zahraa Center for Asthma Allergy through the use of asthma definitions of US National Heart Lung and Blood Institute (NHLBI) 1997 Asthma Management guidelines (11).

METHOD

Study Design: Case-control study depend on comparison between cases (asthmatic patients) and control (apparently healthy). A total of 100 patients and healthy subjects were studied, 50 asthmatic patients compared with 100 healthy subjects.

Study Place: The study was conducted in Chest Diseases Department, Al zahraa Center for Asthma Allergy in Al-Karch directorate in Baghdad city

Study Duration: The study duration was over the period extended from the 1st August 2017 to 1st of

April 2018 .

Sampling Design : All asthmatic patients (26 females and 24 males) who attended Chest Diseases Department, Al zahraa Center for Asthma Allergy in Al-Karch directorate in Baghdad city in the study settings during the study period were included. Their ages ranged from 6 to 73 years.

Inclusion criteria : All asthmatic patients who attended to the study center . All age groups, and both gender

Exclusion criteria : They had to have no history of cigarette smoking and no evidence of primary restrictive lung disease were excluded

The Control group : Apparently healthy control group attending the study center. Cases and controls were collected from the center at the same day.

Sampling methods : Asthmatic patients constitute the target population of the study. The collection of the cases was done by working for about five hours per day for four days per week during the study period.

Data Collection : Data collection was done after getting the verbal agreement from the asthmatic patients to asking them by direct interview through the. The questionnaire was prepared depending on literature of previous studies, The target control to case ratio was 1 to 1. Data collection for cases was done through four days in the week and spending about four hours per day. The main items included in the questionnaire form are : gender , family history of asthma, weight, height .

Classification of asthma severity based on symptoms and asthma therapy as recommended. Asthma control was assessed according to the criteria of the Global Initiative for Asthma to identify controlled, partially controlled, or uncontrolled asthma. Adults who had a diagnosis of asthma made by a pulmonologist were included in the study. All subjects were subjected to the following:

- Complete medical history.
- Routine laboratory investigations.
- Spirometry: performed for each subject (forced vital capacity (FVC), forced expiratory volume in 1(FEV1) , FEV1/FVC ratio) using Geratherm Respiratory GmbH Blue Cherry V1.2.2.1 with a built-

in computer. Each subject performed at least three spirometry maneuvers and the highest values were chosen. The test was repeated 20 min after inhalation of 4 puffs of salbutamol. Each puff contains 100 mcg salbutamol.

– Body mass index BMI was calculated for asthmatic patient and apparently healthy control group. Weight (kg) and height (meter²) was measured by using suitable Scales . Children and teen were classified into subgroups according to their BMI(12).

Weight Status Category	Percentile Range
Underweight	Less than the 5th percentile
Healthy weight	5th percentile to less than the 85th percentile
Overweight	85th to less than the 95th percentile
Obese	Equal to or greater than the 95th percentile

Adults were classified into subgroups according to their BMI(13).

Category	BMI (Kg/m ²)
Under weight	< 18.5 kg/m ²
Normal weight	18.5-24.9kg/m ²
Over weight	25-29.9kg/m ²
Obesity	≥ 30kg/m ²

Statistical Analysis : The information for each case transferred into code sheet and data entry was done using computer and statistical analysis was done using SPSS(Social Package of Social Science) package version 24 the approach to data consists of descriptive and analytic statistics . Chi-square test (X²); this statistical method was used to determine the significant statistical association between variables with results being considered as statistically significant when a p-value was (< 0.05).Fisher exact was used when Chi-squared test was not suitable.

Findings

Figure (1) shows that male 52% of asthmatic patients and female 48%.and this in disagreement with

previous study in America, the prevalence of male was 48.6 (14)

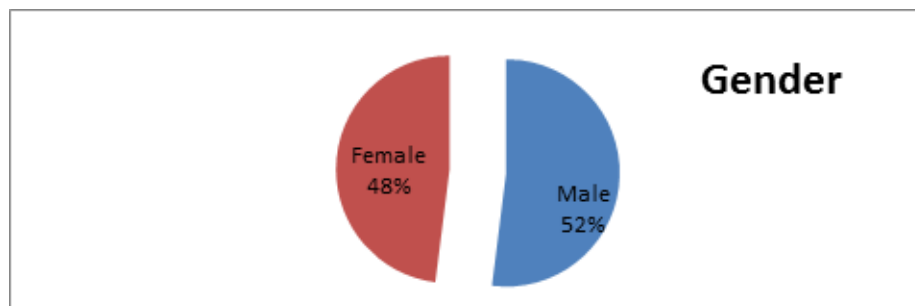


Figure (1) Distribution of asthmatic patients according to gender.

Table (1) shows that in this study the most common age of cases and control was <20 years which represents 68% and 52% of study population respectively and the second highest percentage was 22% and 32% of study population with >40 years respectively, the result recorded that the mean age (22.200 ± 16.2945) was among asthmatic patients, whereas the mean age (28.240 ± 18.9258) among control group. This results was in disagreement with the Turkish study, that indicated the mean age of patient was (43.86 ± 13.18). This study suggested that most patients were young people (15). The disease increase can occur at any age, but develop in adult before the age of 20 years

Table (1) Distribution of Studied Sample according to Age.

Age	Case	Control	Total	p-value		
	No.	%	No.	%		
<20 years	34	68.0	26	52.0	60	0.261
20-40	5	10.0	8	16.0	13	
>40 years	11	22.0	16	32.0	27	
Mean \pm SD (Range)	22.200 \pm 16.2945	28.240 \pm 18.9258				

Table (2) demonstrates the association between asthmatic patients and healthy subjects, cases with history of asthma constitute 54%, while only 5% in control with history of asthma. The association between two groups are highly significant ($p = 0.000$). This finding is incompatible with Olufemi O et al who found that only 7.3% of patients have family history of asthma (16).

Table (2) Distribution of Studied Sample according to family history.

Family history	Case	Control	Total	p-value		
	No.	%	No.	%		
Yes	27	54.0	5	10.0	32	0.000
No	23	46.0	45	90.0	68	

The current study results revealed that 34% of cases and 32% of controls are having a BMI ≥ 30 . While 32% of cases and 22% of controls have normal weight (BMI < 25). In addition, 24% of cases and 42% of controls found having Over weight with no significant finding (P-value = 0.091). Mean of body mass index (BMI) was observed in asthmatic patients (23.85 \pm 6.248) when compared to non- asthmatic subjects (25.68 \pm 5.744). In present study, these findings were lower than that of Hatice Eylül Bozkurt Yılmaz et al, who mentioned that mean of BMI was (29.72 \pm 3.98) asthmatic patients in comparison with healthy control group (15). In addition, the finding of the present study were similar to a previous Turkish study which showed that a non-significant difference (p<0.167) when compared between in asthmatic patients and control group. Table (3).

Table (3) Distribution of Studied Sample according to BMI.

BMI	Case	Control	Total	p-value				
	No.	%	No.	%				
Under weight	5	10.0	2	4.0	7	0.091		
Normal weight	16	32.0	11	22.0	27			
Over wt.	12	24.0	21	42.0	33			
obese	17	34.0	16	32.0	33			
Mean \pm SD (Range)	23.85 \pm 6.248	25.68 \pm 5.744						

From 50 Patient enrolled in current study 39 were uncontrolled asthma (78%) and 11 were controlled asthma (22%). (Figure 2)

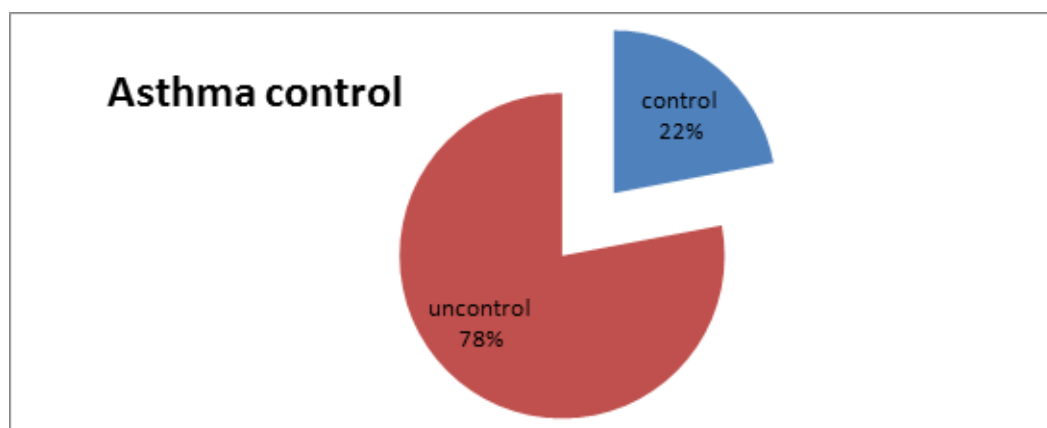


Figure (2): Distribution of asthmatic patients according to asthma control

Data illustrated in table (4) showed the distribution of study groups according to skin test which showed that 47(94%) of cases have positive results. A significant association was found between asthmatic patients when they were compared with that of control groups (p= 0.000). Finding of our study were higher than other study done by S Quirce et al. in Spain . who found 54.2% patients with positive skin test results. In current results of asthma, association was revealed between two groups (p= 0.00)(17).

Table (4) Distribution of Studied Sample according to skin test.

Skin test	Case	Control	Total No.	p-value		
	No.	%	No.	%		
Yes	47	94.0	0	0	47	0.000
No	3	6.0	50	100	53	

Table (5) In this study asthmatic patients who have FEV1 less than 4 were 100%, while control group who have FEV1 less than 4 were 42% . A significant association was found between asthmatic patients when they were compared with that of control groups ($p= 0.000$). Mean levels in the asthmatic patients were ($2.003\pm.2340$) and control subjects were ($3.930\pm.0886$). Similarly, Dr.GurunathBirajdar et al demonstrated significantly difference of FEV1 in asthmatic patients when compared with that in healthy controls(mean 1.23 ± 0.72 of asthmatic patients and mean of control 2.37 ± 0.57)(18).

Also, this table shows the association between two groups in concerning to FVC , asthmatic patients who have FVC less than 5 constitute 100% , while control group who have FVC less than 5 constitute 28% . A significant association was found between asthmatic patients when they were compared with

that of control groups ($p= 0.000$). Mean levels in the asthmatic patients were ($3.576\pm.3668$) and control subjects were ($4.972\pm.0454$). These results do agree with Dr.GurunathBirajdar et al, who found significant difference in asmthatic than in non-asthmatic (mean 1.71 ± 0.78)and 2.59 ± 0.63), respectively, ($p=0.001$)(18).

In this study asthmatic patients who have FEV1/ FVC represent the highest percentage which constitute 100% , while in control group were 42%. A significant association was found between asthmatic patients when they were compared with that of control groups ($p= 0.000$). Mean levels in the asthmatic patients were (55.640 ± 7.5180) and control subjects were ($1.580\pm.4986$).Our study agreed with Erick Forno et al, who reported that significantly difference of FEV1/ FVC in asthmatic patients when compared with that in healthy controls (19).(Table 5).

Table (5): Spirometrical results for asthmatic Patients and Control Group.

Spirometry values	Case	Control	Total	p-value			
	No.	%	No.	%			
FEV1	<4	50	100	21	42.0	71	0.000
	≥ 4	0	0	29	58.0	29	
Mean \pm SD (Range)	$2.003\pm.2340$	$3.930\pm.0886$					
FVC	<5	50	100	14	28.0	64	0.000
	≥ 5	0	0	34	62.0	34	
Mean \pm SD (Range)	$3.576\pm.3668$	$4.972\pm.0454$					
FEV1/FVC ratio	<80	50	100	21	42.0	71	0.000
	≥ 80	0	0	29	58.0	29	
Mean \pm SD (Range)	55.640 ± 7.5180	$1.580\pm.4986$					

Conclusion

Current study shows that asthma is more common in patients under twenty years old. Obesity show not significant decrease in asthmatic patients as compared to healthy control group. Spirometrical changes are common in asthmatic patients. All spirometrical result show highly significant decrease in asthmatic patients as compared to healthy control group.

Ethical clearance: Permission from Chest Diseases Department, Al zahraa Center for Asthma Allergy in Al-Karch directorate were attained before starting the study. All the participants gave verbal consent with confidentiality of participants' identification

Conflict of Interest: Nil

Source of Funding : Nil

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Level of Interleukin -6 and Interleukin -1 1B Associated Bacterial Burn Infections Patients in Al- Kindy Hospital

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Abstract

Background : Burn infections was the most important caused mortality and morbidity in Iraqi hospitals , which caused by gram positive and negative bacteria.

Material and methods collected 151 wound swab and serum from burn patients during the period from January – April 2019 in Al-Kindy Hospital (Baghdad) , all swab were inoculated on (McConkey and blood) agar and incubated aerobically overnight at 37°C ,and identified isolated of bacteria by Vitek system, also determinate the level of both interleukin -6 and interleukin -1 beta by Elisa. Results in current study showed male of burn patients (70.8%) more than female (29.2), as well as control group. Burn patients was more in age group (1-15) as percentage (31.8%) , followed by (15-30) years as (29.2%), but more bacterial isolated in age group (15-30) years as (36.2%), followed by (45≤) years as (25.5%), whilst low percentage (17.0 %) in age group (1-15), Single bacterial isolate (61.5%) more than Mixed bacterial isolate (38.5%), also results of current study showed Gram negative bacteria (74%) more isolated from burn infection than Gram positive bacteria (26%). More bacterial isolate as Single Bacterial isolate were *Pseudomonas aeruginosa* (92.3%), followed both *Klebsiella pneumoniae* and *E.coli* as (18.4 and 18.0) % respectively , whilst bacterial isolate as mixed Bacterial isolate were *Pseudomonas aeruginosa* + *E.coli* (4%) , increased level of Interleukin- 6 in sera of burn infected patients (1.3 ± 0.51) compare to healthy control (0.44 ± 0.20) ($p < 0.001$), whilst no significant ($p > 0.05$) for level of Interleukin-1B in sera of patients (0.70 ± 0.30) and healthy control (0.190 ± 0.019). conclusion Male of burn patients more than female also more bacterial isolated in age group (15-30) years followed by (45≤) years , so single bacterial isolate more than Mixed bacterial isolate. and gram negative bacteria more isolated from burn infection than Gram positive bacteria. as well as more bacterial isolate as Single Bacterial isolate were *Pseudomonas aeruginosa* , followed both *Klebsiella pneumoniae* and *E.coli*, whilst bacterial isolate as mixed Bacterial isolate were *Pseudomonas aeruginosa* + *E.coli* , also increased level of Interleukin- 6 in sera of burn infected patients ($p < 0.001$), whilst no significant ($p > 0.05$) for level of Interleukin-1B.

Keywords : interleukin -6 ; interleukin -1 beta , bacterial burn infections; Al-Kindy hospital.

Introduction

Burn infections is most common causing mortality after burn injury (1;2). Burn infection is an most important problem in many Iraqi hospitals , because the burn wounds can be contaminated with dangerous pathogenic bacteria (gram positive and negative) , and causing mortality and morbidity (3), In the developing countries more than 75% of mortality associated with the

burn infection which related to the the toxemia (4) .most source of contaminated burn wound were endogenous as microbiota (normal flora) or contaminated by exogenous as hospital environment ; dressing devices (5; 6) . Infection can be defined as the presence of high counted (more than 10⁵ bacteria /g of the tissue) in scab and burn wounds , so two types of bacteria can be cause burn infection as both gram (positive and negative) bacteria , but G- Bacteria more causing burn infections (7; 8 ;3) .

Material and methods

During the period from January – April 2019 collected 151 wound swab and serum from burn patients in Al-Kindy Hospital (Baghdad), all swab were inoculated on McConkey and blood agar (Himedia, India), then incubated overnight at the 37°C. Bacterial pathogens were identified by Vitek system. so the determined Level of both interleukin -6 and interleukin -1 beta by Elisa kit (Raybiotech - U.S.A).

Finding :

Table (1) Frequency Burn patients and Control (Healthy) according to gender

Study group	Gender	Total No.(%)			
	Male	Female			
	No.	%	No.	%	
Patients	107	70.8	44	29.2	151(100)
Control (Healthy)	30	60	20	40	50(100)

In this table appearance male of burn patients (70.8%) more than female (29.2%), as well as control group.

Table (2) Frequency of Bacterial isolate according to age of Burn patients

Age group	Burn patients	Bacterial isolated		
	No.	%	No.	%
1-15	48	31.8	8	17.0
15-30	44	29.2	17	36.2
30-45	29	19.2	10	21.3
45≤	30	19.8	12	25.5
Total	151	100	47	100

Burn patients was more in age group (1-15) as percentage (31.8%), followed by (15-30) years as (29.2%), but more bacterial isolated in age group (15-30) years as (36.2%), followed by (45≤) years as (25.5%), whilst low percentage (17.0%) in age group (1-15) (table 2).

Table (3) bacterial isolate from burn infections

Bacterial isolate	No.	%
Single bacterial isolate	8	61.5
Mixed bacterial isolate	5	38.5
Total	13	
Types of bacteria	No.	%
Gram positive bacteria	13	26
Gram negative bacteria	37	74
Total	50	100

Table (3) showed the Single bacterial isolate (61.5%) more than Mixed bacterial isolate (38.5%), also results of current study showed Gram negative bacteria (74%) more isolated from burn infection than Gram positive bacteria (26%).

Table (4) Single and Mixed Bacterial isolate from burn infections

Bacterial isolate	No.	%
Pseudomonas aeruginosa	11	23
Klebsiella pneumoniae	9	18.4
E.coli	7	18.0
Staph.aureus	6	12.0
Coagulase-negative staphylococcus	4	8.4
Acinetobacter baumannii	3	6.2
Enterobacter cloacae	1	2
staph.aureus+ Klebsiella pneumoniae	1	2
staph.aureus +Pseudomonas aeruginosa	1	2
E.coli +Pseudomonas aeruginosa	2	4
Pseudomonas aeruginosa + Klebsiella pneumoniae	1	2
staph.aureus+E.coli	1	2
Total	47	100

More bacterial isolate as Single Bacterial isolate were Pseudomonas aeruginosa (23%), followed both Klebsiella pneumoniae and E.coli as (18.4 and 18.0%) respectively, and Coagulase-negative staphylococcus as percentage (8.4%), whilst bacterial isolate as mixed

Bacterial isolate were *Pseudomonas aeruginosa* + *E.coli*(4%) , while all isolated of *Klebsiella pneumoniae* + *staph. aureus*, *Pseudomonas aeruginosa* + *staph.aureus*, *Pseudomonas aeruginosa* + *Klebsiella pneumoniae* and *staph. aureus*+*E.coli* respectively as (2%)(fig.1).

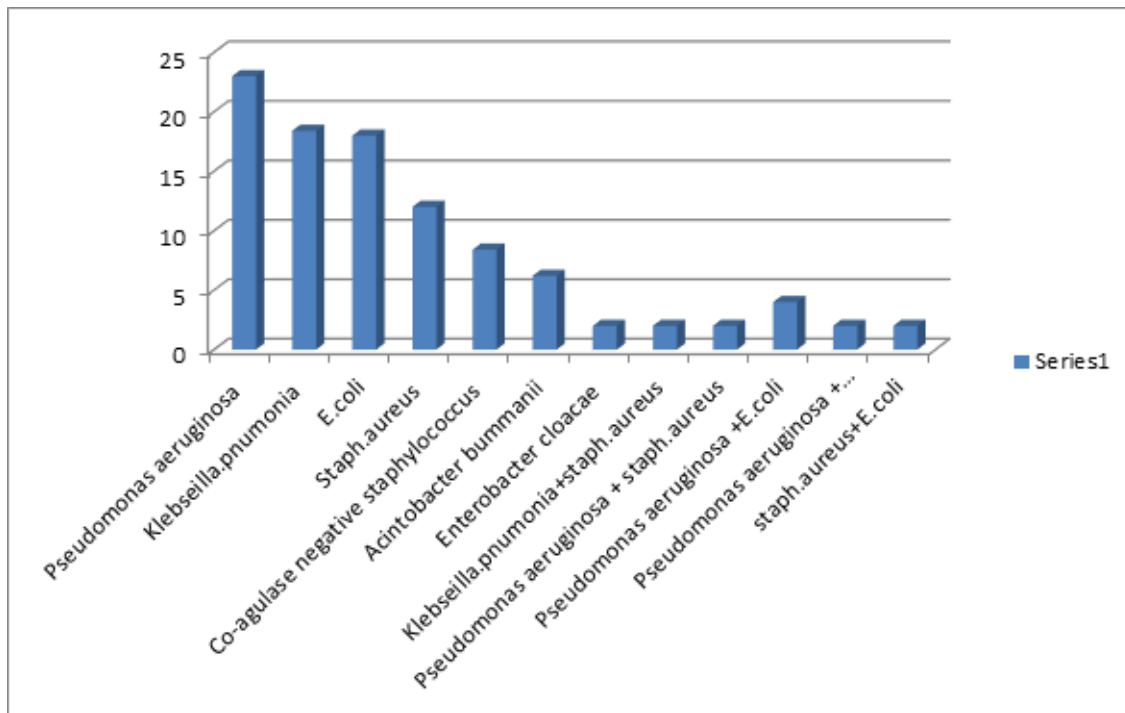


Figure (1): percentage of bacterial isolated

Table (5) level of interleukins in both control and study groups

Interleukin level	Patients (m. ±S.D)	Control (m. ±S.D)	P_value	Significant
IL-6	1.3 ± 0.51	0.44 ± 0.20	p <0.001	High significant
IL-1B	0.70 ± 0.30	0.190 ± 0.019	p >0.05	Non significant

Result of table (5) showed increased level of Interleukin- 6 in sera of burn infected patients (1.3 ± 0.51) compare to healthy control (0.44 ± 0.20) ($p < 0.001$), whilst no significant ($p > 0.05$) for level of Interleukin-1B in sera of patients (0.70 ± 0.30) and healthy control (0.190 ± 0.019).

Discussion :

Current study presented Male of burn patients more than female , Many previous reported , that confirm these results as (9) also (10) who Showed burn infections in Male patients (64.4) % more than females (35.6) % ,as well as Hager et al., 2018 showed high infected in male (64.9) % compare to (26%) female patients (20%)

(16), whilst in India , Rajupt et al., Showed females who have burn infections (60) % more than male (40) % (12), Also in this results showed the burn patients was more in age group (1-15)years ,followed by group (15-30) years, Which correspond to the results of Ghaffar et al., 2002 , who explained found in his study highest percentage of burn infections in age groups (10-20) years (13), But the results of this study came in conflict with Rajupt et al., who showed the lowest percentage (12.2%) of burn infections was found within age group (5-15) years(12).

The current study showed that gram positive bacteria were less isolated than negative gram bacteria from patients of burn infections , as well as more bacterial

isolate as Single Bacterial isolate were *Pseudomonas aeruginosa*, followed both *Klebsiella pneumoniae* and *E.coli*, whilst bacterial isolate as mixed bacterial isolate were *Pseudomonas aeruginosa* + *E.coli*. also in Pakistan study showed incidence of gram positive bacteria less than gram positive bacteria(14;3;15).

The results of the study were fully consistent with (3; 16;17) findings, who showed that most predominant bacteria which isolated from burn infection was *Pseudomonas sp.* bacteria associated with this infections.

Most chief causing burn infection was *Staphylococcus aureus* (18), but *Pseudomonas aeruginosa* is most frequency pathogenic bacteria causing sepsis and leading to burn-linked death (3; 19;20) and can concedes important bacteria causing invasive burn wound invasion(42.6)% and only (11.3) % of cases of burn causing by *Escherichia coli* (21).

Al-Barzinji and Khaleel ,2016Increased level of Interleukin- 6 in sera of burn infected patients compare to healthy control (25), whilst no significant ($p > 0.05$) for level of Interleukin-1B in sera of patients and healthy control (0.190 ± 0.019). The results of this study were consistent with results of the researchers' Hager et al., 2018, who showed all burn infected patients have increased levels of IL-6 (22). Kowal-Vern et al., 1994 explained that increased levels of interleukin - 6 levels as percentage ($> 40\%$) in burn patients with increased size of burn(23), but IL-1 beta appear as poor indicators of prognosis during burn infections(24), this result were confirm with (25) Who showed no significant ($P > 0.05$) in level of (IL-1 β) in burn patients compare to control.

Conclusion

Male of burn patients more than female also burn patients was more in age group (1-15), followed by (15-30) years, but more bacterial isolated in age group (15-30) years followed by ($45 \leq$) years, whilst low percentage in age group (1-15).

Single bacterial isolate more than Mixed bacterial isolate.

Gram negative bacteria more isolated from burn infection than Gram positive bacteria.

More bacterial isolate as Single Bacterial isolate were *Pseudomonas aeruginosa*, followed both *Klebsiella pneumoniae* and *E.coli*, whilst bacterial

isolate as mixed Bacterial isolate were *Pseudomonas aeruginosa* + *E.coli*.

Increased level of Interleukin- 6 in sera of burn infected patients compare to healthy control, whilst no significant ($p > 0.05$) for level of Interleukin-1B in sera of patients and healthy control (0.190 ± 0.019).

Ethical Clearances : The consent of the patient has been taken in the hospital.

Conflict of Interest : Non

Source of Funding : Self

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Anti-inflammatory and Analgesic Activities of the Heart of Date Palm Extract in-vivo

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Abstract

Phoenix dactylifera L or tree of the Date palm is common in North Africa and the Middle East such as states of the Arabian Gulf states including the Iraq. As our knowledge there are no research had studied the anti-inflammatory effect of the ethanol extract of palm tree heart. Therefore, our study focus on an investigating its anti-inflammatory effect in vivo. The anti-inflammatory effect is estimated by using an acute inflammatory model such as egg albumin induced paw oedema and chronic inflammatory model such as cotton pellet induced granuloma as well as detection of total phenols. The current study demonstrated that ethanolic extract of the heart of date palm has a strong anti-inflammatory effect, in both models its exhibited anti-inflammatory effect in a dose and time-dependent manner.

Key words: Heart of Date Palm, Total phenols, Anti-inflammatory, ethanol extract.

Introduction

The inflammation is a complicated biological process response of tissues to invasion by pathogens, antigenic reactions, physical, chemical or traumatic injury. The complicated process of inflammation and inflammatory mediators induce and intensify of many diseases[1]. The non-steroidal anti-inflammatory drugs have great benefits in the treatment of many diseases such as musculoskeletal disorders, osteoarthritis and rheumatoid arthritis and however long use of these drugs causes negative side effects such as fluid retention, nausea, gastric disorder and bleeding[2]. Hence, new anti-inflammatory drugs without side effect are required as an alternative therapy to NSAIDs. Alternative medicine is using many herbs and medicinal plants for treatment of inflammation. Phenolic compounds is one of the essential constituents in many plants responsible for their inflammatory activity. The palm family have (2400) species and (183) genera. Most common palm tree are peach, nut, coconut and date[3]. Phoenix dactylifera L is the scientific name of date palm tree that cultured in many countries in Middle East such as Iraq[4]. Antioxidant effects and phenolic composition were confirmed in several reports[4][5][6]. Movahed et al. [7] reported that the heart of Iranian palm has high nutritional values and it composed from minerals (Fe, Zn, P, Mg, Ca, Mn, Na, and K), crude fiber and

unsaturated fatty acids. Also Shimizu et al. [8] studied the phenolic compounds and polyphenol oxidase activity in the heart of a palm tree. This current study focus on the evaluating the anti-inflammatory and analgesic effect of the heart of Iraqi palm tree in vivo for the first time in country.

Materials and Method

Preparation of Heart of Palm Samples:

Heart of palm tree was provided from the local market in Baghdad, Iraq. The sample is cleaned and removed all depress and freeze-dried. The dried material was pass through 60 mesh sieves and stored in bottle and kept at 4 °C until used.

Sample Preparation:

Freeze-dried samples were filtered by sieved (200 mm) and stored at plastics bags. Preparation of samples was done by adding 200 mg of samples in a beaker with HNO₃ (2 ml), HCl (6 ml) and HF (2 ml). Then the samples were heated on the plate at (110-120) °C for forty minutes. Then filtered by filter paper then transferred to cleaned flask and deionized water was added.

Reagents:

2,2-diphenyl-picrylhydrazyl, Gallic acid and

Folin-Ciocalteu's phenolic were provided from (Sigma company, USA), All reagents were analyzed properly.

Preparation of the Extracts:

Ten gram of extract powder mixed with 100 ml ethanol (75%) with continuous shaking for (24 hr) at (30) °C. The final product were filtered by whatman No. 1 filter paper and the final liquid were analyzed for phenolic constituents, analgesic and anti-inflammatory effect. Samples were analyzed in triplicate.

Total Phenol Determination:

Estimation of the polyphenol compounds were done by using Folin Ciocalteu phenol reagent depends on the protocol of Xu and Chang[9].

Animal:

Swiss albino mice weighed (25-30gm) and adult Wistar rats weighed (150-200gm) of both sexes provided from the faculty of veterinary medicine, Baghdad university. They were housed in metal steel cages and acclimatized in the laboratory for (7) days before the experiment began. Animals had free access to water and food.

Analgesic activity evaluation:

Heat pain test:

The hot plate was used to induce pain in mice and measure of the response latencies[10]. Hot plate was set up at (40±2) °C, the animals were put in a beaker on the heated surface. Furthermore, the time was recorded between the placement and shaking or licking of the paws or jumping as the response latency index. Each 35 seconds, the hot plate were stopped automatically to prevent any damage. The rats were divided into five groups, and six rats for each group then fasted for (24) hour and allowed access to water supply. The first group was the negative control and was given normal saline (1) mL/kg. The second, third and fourth Groups were injected intra-peritoneally (i.p) by the extract (50, 100 and 200) mg/kg, respectively, but the fifth group was given meloxicam (0.6) mg/kg (i.p) 30 minute before putting the animals on hot palate.

Anti-inflammatory test:

Two experiments were used to evaluate the anti-inflammatory effect of the extract. 25 Wistar rats (male

and female) weighed (150-200) gram were randomly distributed into 5 groups, each group had 5 rats. Animals were overnight deprived and fasted from water and food prior the experiments.

Egg albumin-induced paw oedema in rats:

The method of [11][12] were used in our study to induce paw oedema. Three different doses (50, 100 and 200 mg/kg) of Palm heart extract were prepared and injected (i.p) and the dose (0.6 mg/kg) of meloxicam was also prepared and injected i.p. While the control group provided with 1mL normal saline. Inflammation of hind paw was induced by injecting undiluted egg albumin (0.1) ml in the subplanar surface of the right hind paw of rats. The right hind paw volumes of the rats were taken on the principle of volume displacement by using LETICA Digital Plethysmometer immediately before the experiment and at one hour intervals after the injection of egg albumin for five hours. Size of oedema was assessed at every interval based on the differences in volume of oedema after injection of egg albumen and zero time ($V_t - V_0$). Moreover, the percentages of oedema inhibition were also recorded for all treated groups.

Cotton-pellet induced granuloma: The method of Winter and Porter with slight modification was used to study chronic inflammation[13]. The granuloma tissue formation was calculated thus:

$$\frac{T_c - T_t}{T_c} \times 100$$

T_c represents granuloma tissue weight in the control group

T_t represents granuloma tissue weight in the treated group.

Statistical Analysis

The results were expressed as Mean±SEM. Statistical analysis was done using two way ANOVA test using SAS software [14].

Findings

Total Phenols and Flavonoids:

Total phenolic of date palm heart samples were 47.14 (mg GAE/100g extract).

Effect of palm heart extract on thermally-induced pain in mice

The extract showed a dose-dependent effect on heat pain test in mice. Pain inhibition was significant ($P<0.05$) comparing to the control (Table 1).

Table 1: Analgesic effect of Palm heart extract on hot plate test (mean \pm SE)

Groups	Dose (mg/kg)	Reaction time (sec)	Inhibition (%)
Control	1ml/kg	2.25 \pm 0.21d	
Extract	50	5.86 \pm 0.15c	14.30
Extract	100	7.69 \pm 0.12c	25.61
Extract	200	14.45 \pm 0.05b	68.23
Meloxicam	0.6	17.36 \pm 0.11a	92.92%

Values represent mean \pm S.E , n=6

Different small letters mean significant ($P< 0.05$) results between different doses of extract and drug.

Anti-inflammatory effect of the palm heart in reducing the oedema induced by egg albumin

The extract of the heart of palm (50,100 and 200 mg/kg) showed weak anti-inflammatory activity at doses 50 and 100 mg/kg. While the dose 200 mg/kg exert a considerable inhibition of the oedema formation induced by egg albumin when compared with group treated with meloxicam (0.6 mg/kg) which was showed a highest reduction in oedema. Results showed no significant changes in oedema reduction during the hour 1 and 2 except in meloxicam showed an obvious reduction. While the palm heart extract at doses 100 and 200 mg/kg exert considerable reduction in oedema volume after the hour 4 comparing to control group and group treated with 50 mg/kg did not showed any changes during the 5 hours. Table (2) demonstrates the differences between oedema size before and after treatment in all groups.

Anti-inflammatory effect of the palm heart extract in reducing the granuloma induced by the cotton-pellet

The palm heart extract (50, 100 and 200 mg/kg b.w) significantly reduced the cotton pellet induced granuloma tissue formation in the rats. (figure 1). The reduction in granuloma tissue formation recorded for the palm heart extract doses were found to be lower than that obtained for meloxicam (0.6 mg/kg).

Table 2: Effect of the ethanolic extract of palm heart on egg albumin-induced paw oedema in rats.

Treatment	Dose (mg/kg)	Time interval (hr)				
		1 h	2 h	3 h	4 h	5 h
Normal saline (Control)	1 mL/kg	0.45 \pm 0.05 A a	0.58 \pm 0.15 A a	0.62 \pm 0.02 A a	0.55 \pm 0.01 A a	0.42 \pm 0.01 A a
Palm heart extract	50	Paw oedema changes (ml)				
		0.52 \pm 0.02 A a	0.68 \pm 0.04 A a	0.56 \pm 0.03 A a	0.45 \pm 0.02 A a	0.38 \pm 0.04 A a
		% inhibition of oedema				
		21.73	18.81	24.77	27.54	21.14
Palm heart extract	100	Paw oedema changes (ml)				
		0.66 \pm 0.01 A a	0.58 \pm 0.03 A a	0.42 \pm 0.07 A a	0.22 \pm 0.04 B b	0.18 \pm 0.06 B b
		% inhibition of oedema				
		26.08	24.09	30.66	56	58.7
Palm heart extract	200	Paw oedema changes (ml)				
		0.59 \pm 0.03 A a	0.52 \pm 0.05 A a	0.46 \pm 0.04 A b	0.18 \pm 0.05 B c	0.11 \pm 0.01 B c

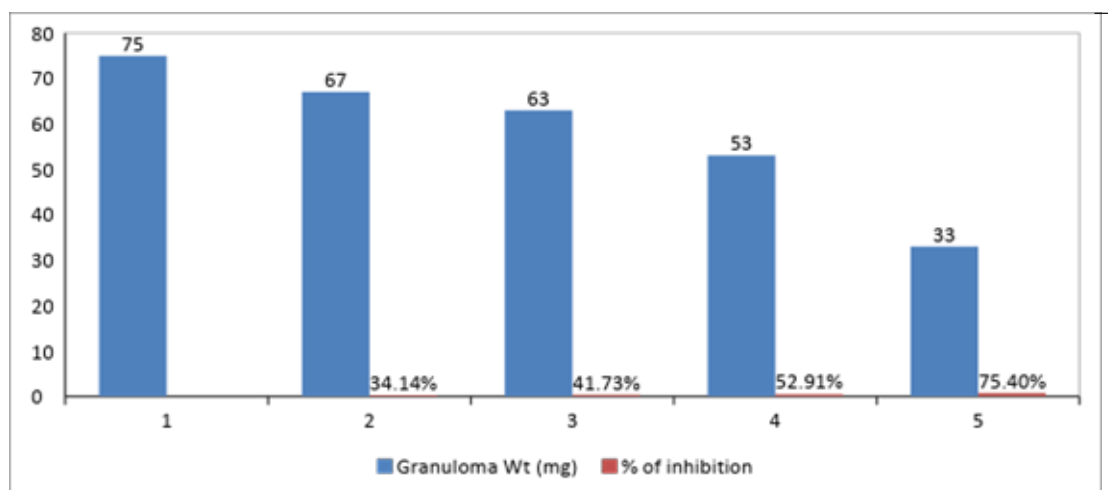
Cont... Table 2: Effect of the ethanolic extract of palm heart on egg albumin-induced paw oedema in rats.

		% inhibition of oedema				
		35.86	40.9	50	67.27	73.8
Meloxicam	0.6	Paw oedema changes (ml)				
		0.62±0.07 A a	0.28±0.09 B b	0.17±0.02 B c	0.14±0.03 B c	0.10±0.02 B c
		% inhibition of oedema				
		32.6	44.09	65.83	72.72	73.9

Values represent mean \pm S.E , n=5

Different capital letters mean significant ($P < 0.05$) results between doses of extract and drug .

Different small letters mean significant ($P < 0.05$) results between hours.

**Figure 1: Effect of the ethanolic extract of palm heart on cotton pellet granuloma.**

Modern researchers focus on research that related to the inflammation because of significant complications of inflammatory diseases in human and animal. The plant could use in the treatment of inflammatory diseases because of its potential great therapeutic and cheapness without a negative side^[15]. Our work focus on an estimation of analgesic and anti-inflammatory effect of palm heart extract by using several models. Based on our knowledge there was no previous reports studied the anti-inflammatory and analgesic activity of the heart of palm tree, and this work may be the first time conducted in Iraq. The heart of the palm tree have great effects due to it contain large amounts of phenolic substances; wherever phenolic compounds have an anti-inflammatory effect ^{[16][17]}. Our study was in an agreement with ^[18] they found that the total

phenol in palm heart extract in Saudi Arabia were 41.44 mg GAE/100g extract. Iranian date palm has high nutritional value as founded by Movahed et al^[17], they demonstrated that the samples contain many minerals, crude fiber and unsaturated fatty acids. Shimizu et al.[8] studied the polyphenol oxidase activity and phenolic profile in heart of palm samples extract. Alternative medicine is using many herbs and medicinal plants for treatment of inflammation. Phenolic compounds is one of the essential constituents in many plants responsible for their inflammatory activity^[3]. The anti-inflammatory action of the ethanol extract of the heart of Iraqi date palm may be related to the phenolic contents. Searching for medicinal plant contain a secondary metabolites with anti-inflammatory activity with minimal side effects such as phenolics have attracted research attention by

research laboratories and pharmaceutical company. This active compounds are caused inhibition of arachidonate 5-lipoxygenase (5-LO) and COX-2 enzymes^[19]. In this perceptiveness, some phenolic compounds have revealed anti-inflammatory activities. Although the exact mechanisms of this anti-inflammatory effect are not totally understandable, there is a relationship between the food rich with phenols and inhibition of the inflammatory processes^[20]. Phenolic compounds have the same mechanism of action as NSAIDs, some of them exceed this function by decreasing the activity and inhibition the gene expression of COX enzyme and other pro-inflammatory mediators. Some phenolic compounds might increase and decrease transcriptional factors such as (Nrf-2) or (NFkB)^{[16][17]}. Furthermore, our study demonstrated the high similarity between the effects of paracetamol which have antipyretic and analgesic effects by inhibition prostaglandin synthesis in the brain by interfering with action of COX-3^{[21][22]}. Yet it doesn't show activity on peripheral prostaglandin^[23]. The heart of tree palm extract act centrally which is supported by its effectiveness to inhibit both phases of oedema induced by egg albumin, which is a characteristic of drugs (such as narcotics) that act centrally^[24]. Moreover, the ethanol extract of the palm heart may have anti-nociception activity which is interpret the analgesic effect of the extract. Furthermore, another explanation that the extract has central action is by elongate the response time to heat and this may attributed to involvement of narcotic or opioid receptors^[25].

Conclusion

This current study showed that the heart of date palm was rich in polyphenols and can be used as anti-inflammatory and analgesic. Also it demonstrate that the ethanol extract of the heart of Iraqi date palm tree revealed significant anti-inflammatory activity, In both model its showed anti-inflammatory effect in a dose and time dependent manner. Further study are required to understand the precise mechanism and sites in which phenolic compound provide anti-inflammatory and analgesic activities.

Conflict of Interest: Authors have no conflict of interest.

Source of Funding: This research is a personal non-profit work.

Ethical Clearance: The protocol was approved by the Animal Care and Use Committee of the University of Baghdad, College of Veterinary medicine prior to the initiation of the study. (no. 219, 2/6/2018).

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Nursing Students' Knowledge about Risk Factors of Infection among Patients with Hematologic Disorders at College of Nursing, University of Baghdad

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Abstract

People may be affected by many different types of blood conditions and blood cancers. Approximately every 4 minutes, someone in the United States is diagnosed with a type of blood. Furthermore, as stated by the European Hematology Association (EHA), an estimated 80 million people are currently affected with blood disorders in the European Union (EU). This study aims to assessing the nursing students' knowledge about the risk factors of infection among hematologic disorders patients, in which an approach of assessment was applied to achieve that. A descriptive (cross-sectional) study was conducted during 3rd November, 2017 to 3rd May, 2018. A purposive (non-probability) sample of 100 students from the forth academic stage was involved. Data were collected through the use of the questionnaire format by using the self-administrative approach with those students from both morning and evening studies. Data were analyzed through the application of descriptive and inferential statistical approaches by using Statistical Package for Social Science (SPSS) version 20.0. More than half of students were males; the highest percentage of the study sample was aged twenty one years old, the majority of the students who participated in the study were from the morning and they were not working. More than half of them were living in dorm room. Severity of mean score for students' level of knowledge was moderate as general. The males, morning study, the students not working besides their studying, and living in a dorm room were the major dominates of the sample with a moderate knowledge level related to identifying risk factors of infection for patients with hematologic disorders.

Keywords: Risk factors, Infection, Hematological Disorder.

Introduction

People may be affected by many different types of blood conditions and blood cancers^[1]. Approximately every 4 minutes, someone in the United States is diagnosed with a type of blood cancer^[2]. Furthermore, as stated by the European Hematology Association (EHA), an estimated 80 million people are currently affected with blood disorders in the European Union (EU)^[3]. Consistently, blood cancers (e.g. Hodgkin's lymphoma, non-Hodgkin lymphoma, multiple myeloma, and leukemia) are one of the 10 most common forms of cancer and are responsible for approximately 100,000 deaths in Europe every year^[4]. Common blood disorders include anemia, bleeding disorders such as hemophilia, blood clots, and blood cancers such as leukemia, lymphoma, and myeloma^[1]. Human

beings' blood is living tissue made up of liquid and solids. The liquid part, called plasma, is made of water, salts and protein. Over half of your blood is plasma. The solid part of your blood contains red blood cells, white blood cells and platelets^[5]. This study aims to assessing the nursing students' knowledge about the risk factors of infection among hematologic disorders patients, in which an approach of assessment was applied to achieve that.

Material and Method

A descriptive (cross-sectional) study was conducted during 3rd November, 2017 to 3rd May, 2018. Assessing the nursing students' knowledge about the risk factors of infection among hematologic disorders patients was the main study objective, in which an approach

of assessment was applied to achieve the mentioned objectives. The validity was determined through the use of panel of (12) experts, they were faculty members from College of Nursing\ University of Baghdad to investigate the clarity, relevancy, and adequacy of the questionnaire. The experts' suggestions were taken into consideration and modifications were employed and the final constructed instrument was completed for conducting study. The reliability was determined through the use of Test- retest through the computation of Pearson Correlation Coefficient ($r = 0.83$). The students' knowledge concerning risk factors of infection for patients with hematologic disorders questionnaire consisted of two sections: the first contained their socio-demographic characteristics: gender, age, type of study, academic stage, working, and residence; the second part contained 19 items focused on the students' knowledge for risk of infection. These items were rated based on the following range (1-3). Whereas "1" stands for "I don't know", "2" stands for "not sure", "3" stands for "I know".

A purposive (non- probability) sample of 100 students from the forth academic stage was involved. Data were collected through the use of the questionnaire format by using the self-administrative approach with those students from both morning and evening studies. Each student takes time from 10 – 15 minutes to fill the questionnaire. Data were analyzed through the application of descriptive and inferential statistical approaches by using Statistical Package for Social Science (SPSS) version 20.0.

Findings and Discussion

Table1. Socio-demographic Characteristics of the study sample

Gender	Frequency (F)	Percent (%)
Male	51	51.0
Female	49	49.0
Total	100	100.0
Age (year)		
20	2	2.0
21	29	29.0
22	23	23.0
23	27	27.0

Cont... Table1. Socio-demographic Characteristics of the study sample

24	5	5.0
25	12	12.0
26	1	1.0
32	1	1.0
Total	100	100.0
M. ± S.D. 22.54 ± 1.6		
Academic Stage		
Forth	100	100.0
Total	100	100.0
Study Type		
Morning	65	65.0
Evening	35	35.0
Total	100	100.0
Working		
Yes	16	16.0
No	84	84.0
Total	100	100.0
Residence		
With Family	46	46.0
Dorm Room	54	54.0
Total	100	100.0

As presented in table (1), the results revealed that more than half of the samples were male student and (29%) of the study sample is frequently seen who age are twenty one years old. The entire study samples (100 %) were from the forth academic stage.

The students' sample who shared in the study was from the morning study rather than the evening, and the majority (84%) of them weren't working. While living in a dorm room formed a rate of (54%) from the study samples rather than living with their families.

Table 2. Level of Nursing Students' Knowledge with Their Socio- demographic Characteristics

Variable Low		Level of Nursing Students' Knowledge toward Risk Factors of Infection among Patients with Hematologic Disorders			Total
		Moderate	High		
1. Age (year)	20	0	1	1	2
	21	0	22	7	29
	22	0	17	6	23
	23	1	21	5	27
	24	0	4	1	5
	25	1	10	1	12
	26	0	1	0	1
	32	0	1	0	1
Total		2	77	21	100
2. Gender					
Male		1	41	9	51
Female		1	36	12	49
Total		2	77	21	100
3. Study Type					
Morning		1	47	17	65
Evening		1	30	4	35
Total		2	77	21	100
4. Working					
Yes		1	14	1	16
No		1	63	20	84
Total		2	77	21	100
5. Residence					
with Family		1	33	12	46
Dorm Room		1	44	9	54
Total		2	77	21	100

Table (2) results demonstrated the characteristics of the study sample for both morning and evening studies. The (21) years old students have moderate level of knowledge higher than other ages, who identify the risk factors of infection for hematologic disorders patients. In spite of moderate level of knowledge to identifying the risk factors, the highest rates were from males rather than females. This indicating the males is better able to

identifying the risk factors than females concerning the study subject.

The morning study sample represented the highest responses to identifying the risk factors rather than the evening study sample; those have a moderate level of knowledge. Not working besides the study, for both studies was formed more than half of the sample having

moderate level of knowledge. A forty four percent of the students living in a dorm room rather than livings with their families have moderate level of knowledge too.

Conclusions

1. More than half of students were males, which indicate the students of the forth academic stage were males more than females.

2. The findings of the study reflecting a moderate level of students' knowledge concerning the risk factors of infection for hematologic disorders patients.

3. The results appear that the students aged twenty one years old who representing the most ages, having a moderate level of knowledge.

Source of Finding: Self

Ethical Clearance: Non

Conflict of Interest: Non

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Antiuro lithiatic Activity of the Plant Extracts Of *Annona* on Ethylene Glycol Induced Urolithiasis in Rabbits

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Abstract

Ethylene glycol (EG) is a colorless, odorless, sweet-tasting chemical mainly used as antifreeze which is fatal if ingested. *Annona* is used as spices and as an herbal medicine (antioxidant) in America countries. Aim of the research was to evaluate the protective role of *Annonaceae* against the Ethylene glycol as toxicity in rabbits. Twenty five rabbits were divided into five groups: (GI, control group) 5 rabbits untreated with Ethylene glycol and (GII) 5 rabbits were given 0.75 % Ethylene glycol in drinking water only for 30 days, while (GIII, GIV and GV) 15 rabbits were given 0.75 % Ethylene glycol in drinking water and oral supplementation of *Annona* (flavonoids, glycosides and alkaloids) extracts (100mg /kg Bw) two times daily for 30 days. Blood samples were collected (plain tube & EDTA tube) to clinical examination. EG induced significant reduction (20%) in rabbits BW in G II in comparison with GI, GIII, GIV and GV. The result shows, AST, ALT, ALKP, TBIL, MDA, SOD, urea, creatinine, globulin and monocytes in G II were significantly elevated ($P=0.05$); meanwhile there were significant decrease ($P=0.05$) in total protein, albumin, A/G ratio, Gpx, CAT, GSH, BP, Hb, WBC and RBC. But after treatment by extracts of *Annona* significant reduction in AST, ALKP, TBIL, MDA, urea, creatinine and monocytes, while SOD, ALT no significant decrease. Meanwhile there were significant increase ($P=0.05$) in total protein, A/G ratio, Gpx, GSH, BP, Hb, WBC and RBC.

Keywords *Annona*, Ethylene Glycol, Toxicity, Blood, Liver and kidney marker enzymes, Antioxidants, Oxidative stress.

Introduction

After the technological developments in the present medical practice, the formation and growth of renal calculi continues to afflict humankind. Though various kinds of stones have been identified, calcium stones are the most common in human as well as in rabbit⁽¹⁾. Urolithiasis is a common disorder estimated to occur in approximately 12% of the world population, with a recurrence rate of 70-81% in males and 47-60% in females. The synthetic drugs used to prevent urolithiasis are not effective in all patients, and many of them have adverse effects that compromise their long-term use⁽²⁾.

In addition to the traumatic effect of shockwaves, persistent residue stone fragments and the possibility of infection suggests that ESWL may cause acute renal injury, a decrease in renal function and an increase in stone recurrence⁽³⁾. Hence, the search for effective antilithiatic drugs without or minimum side effects from natural sources has gained a great potential. *Annona muricata* L.,

commonly known as soursop, and sirsak, is a member of the *Annonaceae* family comprising approximately 130 genera and 2300 species⁽⁴⁾. *Annona* used as traditional medicines against an array of human ailments and diseases, especially cancer and parasitic infections. The fruit is used as natural medicine for arthritic pain, neuralgia, arthritis, diarrhea, dysentery, fever, malaria, parasites, rheumatism, skin rashes and worms, and it is also eaten to elevate a mother's milk after childbirth. The leaves are employed to treat cystitis, diabetes, headaches and insomnia, whereas the cooked leaves are topically used to treat abscesses and rheumatism⁽⁵⁾. Extensive phytochemical evaluations on different parts of the *Annona* plant have shown the presence of various phytoconstituents and compounds, including alkaloids, megastigmanes, flavonol, triglycosides, phenolics⁽⁶⁾, cyclopeptides and essential oils⁽⁷⁾. However, *Annona* species, including *A. muricata*, have been shown to be a generally rich source of annonaceous acetogenin compounds⁽⁸⁾. The presence of different major minerals such as K, Ca, Na, Cu, Fe and Mg suggest that regular

consumption of the *A. muricata* fruit can help provide essential nutrients and elements to the human body ⁽⁹⁾.

Material and Method

Plant material: *Annona* was purchased from the local market. It was classified according to plant classification references related to medicinal plants ⁽¹⁰⁾. Also a vouchers specimens of the plant was identified and authenticated at the herbariums of the College of Education, University of Mosul.

Preparation of extracts: Preparation of flavonoids, glycosides and alkaloids extracts of *Annona* were done according to the method described by ⁽¹¹⁾.

Animal grouping: Male locale rabbit's weightings between 750-850 gm were used, animals were divided into five groups, each containing three animals. Group I animals served as normal control and maintained on regular laboratory diet and water ad libitum. Group II to V animals were fed with 0.75% ethylene glycol (EG) in water to induce renal calculi till 30th day ⁽¹²⁾. Group III to V animals were served as curative regimen and received flavonoids, glycosides and alkaloids extract of the plant of *Annona* at a dose of 100 mg/kg body weight from 15th day to 30th day. The extracts was administered two daily by oral route.

Hematological Assays: The EDTA blood samples were employed for measurement of red blood cell (RBC) count, white blood cell (WBC) count, platelet (PLT) count, hemoglobin (Hb) concentration and hematocrit percentage (Ht%) by using an automated hematology analyzing system.

Liver and Kidney function tests: the serum was extracted from each blood sample for spectrophotometric determination levels of gamma-glutamyltransferase (GGT) ⁽¹³⁾, aspartate transaminase (AST) & alanine transaminase (ALT) ⁽¹⁴⁾, alkaline phosphatase (ALP) ⁽¹⁵⁾, total bilirubin (TBIL) ⁽¹⁶⁾, total protein & albumin ⁽¹⁷⁾, the globulin values and Albumin/globulin ratios were calculated, urea ⁽¹⁸⁾ and creatinine ⁽¹⁹⁾.

Estimation Lipid peroxidation and antioxidant enzyme in serum.

Estimation the levels of malondialdehyde (MDA) by using Thiobarbituric acid reactions method. Thiobarbituric acid (TBARS) in the serum was estimated by the method of ⁽²⁰⁾ & estimating the GSH by the method of ⁽²¹⁾. Antioxidant enzyme activities (CAT, SOD and GPx), the activities were assayed by the methods of ^(22,23) and ⁽²⁴⁾ respectively.

Findings

The toxic EG in (GII) resulted in 20% mortality rabbit (1/5) in rabbits over the study period. However, when extracts of *Annona* (flavonoids, glycosides and alkaloids) were concomitantly administered with EG, it fully protected the rabbits from the acute lethal effect of EG and no mortality rabbit was recorded in G III, IV and V.

Table 1 showed that rabbits subjected to the EG alone (GII) developed significant liver, kidney damage as evidenced by a significant elevation ($P=0.05$) in serum activities of AST, ALT, ALK PH, TBIL, urea, creatinine, total protein and calculated globulin levels. Meanwhile there was significant decrease ($P=0.05$) in albumin, and A/G ratio compared to the corresponding control GI and treated group (GIII GIV & GV)

Table 1: Changes in liver & kidney function tests ethylene glycol group and treated ethylene glycol group as compared to control.

Parameters	Control	EG group	EG group with flavonoid	EG group with glycoside	EG group with alkaloids
AST (U/L)	30.13±0.41	61.33±0.32*	25.95±1.97	35.95±1.37	25.25±1.97
ALT (U/L)	35.66±1.2	52.19±2.02*	53.22±1.76*	53.32±1.76*	49.12±1.26 *
GGT (U/L)	100±4.3	210±11.5*	154±3.9*	167±4.8*	159±5.6*
ALKP (U/L)	31.81±0.78	67.11±1.13*	45.73±1.11	25.73±1.99	39.73±1.09

Cont... Table1: Changes in liver& kidney function tests ethylene glycolgroup and treated ethylene glycol group as compared to control.

TBIL (mg/dL)	87.23±1.51	195.61±2.45*	95.57±4.32	93.57±4.01	111.57±2.32
urea(mg/dl)	18.95±0.805	38.17±1.02*	22.68±0.419	33.55±2.325 *	37.01±1.24*
creatinine(mg/dl)	2.283±0.08	4.133±0.16*	2.13±0.13	4.33±0.20 *	2.313±0.19
Total Protein (g/dL)	10.72±0.03	12.46±0.11*	11.79±0.10	10.19±0.10	10.79±0.11
Albumin (g/dL)	6.35±0.03	5.79±0.55*	8.67±0.41	8.27±0.42	6.67±0.42
Globulin (g/dL)	3.57±0.05	4.44±0.23*	4.54±0.12*	4.44±0.12*	4.51±0.11*
A/G Ratio	2.78±0.04	1.31 ±0.23*	1.90±0.20	1.85±0.12	1.44±0.12*

Blood samples were taken after 30 days of oral administration, number of rabbit each group = 5 Values are given as mean± SD. * means P value <0.05 = significant level

As illustrated in Table 2, there was a remarkable increase in the number of monocytes; while, decrease in: the count of WBCs, RBCs, & platelets, HB%, and, hematocrit value in blood of EGgroup (GII) However, treatment of theseEGreceived rats with crude extract of annonaceae (GIII, GIV,GV) significantly reversed these findings.

Table 2: Changes in hematological tests of ethylene glycolgroup and treated ethylene glycol group as compared to control.

Parameters	Control	EG group	EG group with flavonoid	EG group with glycoside	EG group with alkaloids
Monocytes	0.20 ±0.11	0.49 ±0.17*	0.37±0.2*	0.39±0.2*	0.41±0.2*
White blood cells count (103/uL)	6.01±0.33	2.41±0.11*	5.45±0.21	4.66±0.2	5.71±0.2
Red blood cells count (103/uL)	6.42±1.2	3.34±0.56 *	5.07±1.1	5.71±1.3	6.19±1.0
Blood Platelets (103/uL)	155±15	75±8.3*	128±11	120±14	128±14
Hb concentration (g/dL)	14.1±1.1	5.5±1.11*	12.1±2.2	12.1±2.1	11.1±1.1
Hematocrit value (%)	40.7±2.2	15.5±1.3 *	34.21±1.1	32.23±2.1	34.71±1.1

Blood samples were taken after 30 days of oral administration in EDTA tube, number of rabbit each group = 5 Values are given as mean± SD. * means P value <0.05 = significant level

Table 3 shows that the levels of lipid peroxidation indicated by TBARSweresignificantly higher in Serum of EG group as compared with normal subjects. Also the result showed asignificantlyelevate in GSH levelin the serum of rabbits.Further, the activity of SOD, CAT and

GPx were significantly lower than the normal subjects were treatment of EG groupwith *Annona* significantly elevated the antioxidant defense activity compared with that before treatment.

Table3: Changes in serum lipid peroxidation and enzymatic antioxidants of ethylene glycol group and treated ethylene glycol group as compared to control.

Parameters	Control	EG group	EG group with flavonoid	EG group with glycoside	EG group with alkaloids
MDA($\mu\text{mol/L}$)	0.42 \pm 0.02	0.89 \pm 0.02*	0.65 \pm 0.03	0.87 \pm 0.03*	0.48 \pm 0.02
GSH($\mu\text{mol/L}$)	75 \pm 3.21	47 \pm 2.1*	78 \pm 3.1	69 \pm 3.3	61 \pm 2.1
CAT (U/ML)	0.17 \pm 0.01	0.110 \pm 0.01*	0.112 \pm 0.02*	0.119 \pm 0.02	0.116 \pm 0.02
GPx(U/ML)	5.82 \pm 0.21	3.11 \pm 0.32*	5.45 \pm 0.42	5.43 \pm 0.49	5.98 \pm 0.49
SOD	2.22 \pm 0.2	1.43 \pm 0.2*	1.87 \pm 0.12	1.41 \pm 0.13*	1.56 \pm 0.12*

Blood samples were taken after 30 days of oral administration, number of rabbit each group = 5 Values are given as mean \pm SD. * means P value <0.05 = significant level

Discussion

We have designed the study to investigate the effect of *Annona* extracts (flavonoids, glycosides and alkaloids) supplementation on the development of EG over dose intoxication and its associated life-threatening sequelae. Interestingly, our data showed that concomitant administration of *Annona* successfully prevented the acute lethal effect of EG toxicity and protected rabbit liver and kidney from the destructive effects of EG overdose intoxication. The different bio-chemical parameter registered a significant raise in serum of ethylene glycol treated Group-II rabbits as compared to the normal control Group and *Annona* extracts treatment. In the present study was designated to document the toxic effects of exposure to ethylene glycol on rabbit livers, kidney and possible protective role of *Annona*. Administration of EG led to weight loss and ultimately death of the some rabbits. *Annona* antioxidant effect against chemical induced hepatotoxicity had been approved with previous study⁽²⁵⁾. Abnormalities in liver function indices with EG had been reported in the current study were in accordance with other studies that recorded a progressive elevation in ALT, AST, GGT enzymes, and protein concentrations. In agreement with these results: Gunathilake, et al. 2014⁽²⁶⁾; who found that Ginger antioxidant and protective role against organ toxicity. Among our interesting findings in this study are the hematological results, whereas there was a remarkable decrease in the number of WBCs, RBCs, and blood platelets in the blood of rabbits with ethylene glycol overdoses; and also they were associated with

significant decreases in total hemoglobin and hematocrit that associated also with increase of monocytes count. This was in agreement with Starek, et al. 2012⁽²⁷⁾. However, treatment of these rabbits with extracts of *annona* significantly prevents these hematological toxic effects of ethylene glycol as proven by Samira, 2013⁽²⁸⁾. In harmony with our findings, a study recorded the same fact in mice and the authors found that many chemicals as ethylene glycol in its overdose liberates some of the toxic products that have powerful DNA destructing effects on bone marrow DNA and so decrease of all blood elements as well as that reactive oxygen species (ROS) play a major role in the progression of disease⁽²⁹⁾. Substances that can attenuate the production of ROS, such as *Annona muricata*, can potentially slow or stop the progression of disease. A recent Iranian study has also recorded the same fact in rats, and the authors found that overdose of ethylene glycol resulted in producing some of the toxic agents that have powerful destructing effects on renal. Interestingly, this damaging effect disappeared in *Allium Jesdianum* treated rats⁽³⁰⁾. In agreement with these results: Huang et al. 2002⁽³¹⁾; Oxidative damage as reflected from increased level of marker of oxidative injury by higher MDA and decreased antioxidant enzymes activity like SOD, GPx, CAT and GSH level in the serum as well as deteriorate since kidney functions as observed in calculi induced rabbits. While treatment with *Annona* extracts due to decrease MDA level and increase the activity of antioxidant enzymes and level of GSH indicate that it protected against oxidative stress induce tissue damage.

Conclusion

Annona might be more effective in amelioration of ethylene glycol induced toxicity.

Conflict of Interest :None

Source of Funding :Self

Ethical Clearance: All samples taken after the patients' approval.

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Therapeutic and Identification Study of Tinea Capitis Infection in Basra City

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Abstract

The current study included isolation and identification of some types of dermatophytic fungi that causes Tinea capitis in the province of Basrah from the patients of the Basra General Hospital and a private clinic in the province. The results showed that the number of positive samples of the culture was 110 samples and 61.11% as cases of Tinea capitis, as the number of male infected 78 samples and 70.90%, while the number of female infected was 32 and 29.09%, and found that the age group 6 - 10 years is more infection to develop tinea capitis and 47.27%,. The dermatophytes that causing tinea capitis were diagnosed using a number of diagnostic tests. *Microsporum canis* was found to be more frequent by percentage 49.09%,

The other side of the study was to test the inhibitory activity of clove extracts and peel of pomegranate in *M. canis*, using Agar Well Diffusion Method by using several different types of polar solvents. The results of the study showed that the Hexane extract of the clove plant had the highest effect in *M. canis*,. A comparison of Griseofulvin was found to be the most effective fungal antifungal against *M. canis*,. GC-MS has been used to diagnose fatty acids for the hexane extract of cloves..

Keywords:- Tinea capitis , Microsporum canis , dermatophytes

Introduction

Dermatophytic infections in the head area are known as Tinea capitis, which affects the scalp and hair^[1], also known as the scalp ring worm. This type of infection are found mainly in children, although they are observed at all stages of life and have a higher incidence of male infection than in females^[2]. The infection occurs in both the genus *Microsporum* and *Trichophyton*^[3]. The main cause of this infection is Zoophilic dermatophytes, *M. canis*, *T. mentagrophytes*^[4]

The treatment of fungal infections of various types is very difficult and dermatophytosis is one of the most common fungal diseases and one of the most common skin diseases in the world, as 10% of the world's population is infected with dermatophytosis^[5]. The methods that can be used in this study to treat tinea capitis are the use of antifungal, because fungi are eukaryotic organisms, they have a structure and metabolic processes similar to humans so the antifungal as well as their ability to cause damage in pathogens, Affect the tissues of the human body^[6]. The fungicides used in the treatment of fungal

infections are divided into several types: Azoles, which include Imidazole, Triazole, Polyenes, Allylamines, and Miscellaneous^[7].

WHO has confirmed that nearly 80% of human beings use medicinal plants to treat various types of bacterial and fungal infections^[8]. The herbal drug is characterized as the substance of efficacy and safe when used, which is economical and low cost^[9]. Many fungal isolates have shown resistance to antifungal agents, so plant extracts have been used as therapeutic alternatives to these antifungal^[10]. In this field, many studies have been carried out that have shown the importance of using plant extracts, Various types of medicinal plants have been used. Their extracts have been tested and their biological activity has been tested, The plants used in this study are *Syzygium aromaticum*. The clove of plants belongs to the family of the Asian Myrtaceae^[11].

Cannophyllin is an essential ingredient for clove. Eugenol is the main compound of cloves. It contains 85%^[12]. Eugenol is characterized by anesthetic and sterilized, especially in dentistry^[13].

hexane, ethyl and mixture extractsofpomegranate and clove at a concentration of 700 mg / ml and then determine the minimum inhibitory concentration, using concentrations, 700,500,200,100,50 mg / ml for each plant extract .

Statistical Analysis

The results were statistically analyzed using the SPSS using the Chi-square (X²) and ANOVA (Analysis of Variance Table) and the Least Significant Difference (LSD) using the absolute random design to compare the averages below the level Probability 0.05> p ^[20].

Findings

The results obtained during the present study showed that 110 samples of 180 samples and 61.11%percentage were positive for culture as Tinea capitis. The number of male infections was 78 and 70.90% percentage . The number of female infected was 32 and 29.09% percentage The results of this study showed that male with tinea capitis had the highest percentage of females. The number of males with ringworm was 78 and 70.90% percentage , while the number of female infections was 32 and 29.09% percentage as in Figure 1.The results of statistical analysis have a significant difference P <0.05, X² = 17.48.The results showed that the age group 6-10 years was more likely to develop tinea capitis and 47.27% percentage, followed by the age group 1 - 5 years and 40.00% percentage and was the least age group infection is the largest group of 15 years, the incidence rate was 0.90 %. A significant difference was found between the age groups covered by the study X² = 17.03834, P <0.05.

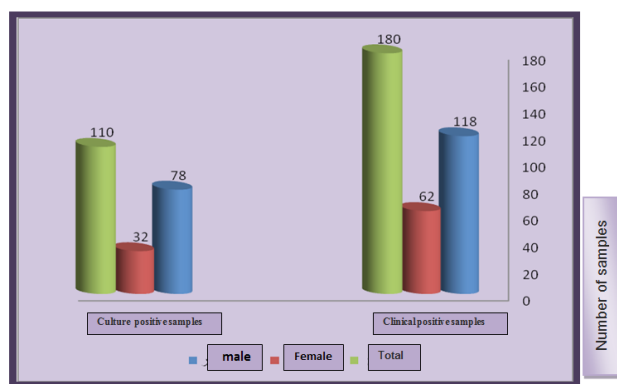


Figure (1) Samples and their positive appearance as head scarf infections

Tinea Capitis according to sex and age:

The results showed that male with ringworm had the highest percentage of females with tinea capitis 78 and

Punica granatum was also used in this study, The pomegranate plant belonging to the Punicaceae family is one of the plants that were used for medicinal, and other purposes ^[14].The tanin material are two types: condensed tannin or biodegradable tannins, which have the solubility of water ^[15].Pomegranate peel contain phenolic substances , It is used in tanning and is used as a disinfectant for its high efficiency in killing microorganisms^[16].

Materials and Method

A total of 180 samples were collected from people with tinea capitis disease from the dermatology at Basra General Hospital. The study included 118 male samples, 62 female samples and age groups ranging from 6 months to 42 years. The sex, age, The hair samples were collected from head hair using scalpel sterile medical The samples were then brought to the laboratory for examination and transplantation. The hair samples were culture onSaproid Dextrose agar with the addition of Chloramphenicol and Cycloheximide (Actidione) incubated at 27 ± 2 ° C ^[7].

Preparation of water extracts:

According to ^[17].and ^[18].method were used to prepare the water extracts by adding 10 g of dry plant powder to each plant and full size to 100 ml distilled water in a 500 ml glass flask and put the mixture in an electric mixer for 15 minutes and then leave The solution for 30 minutes for the purpose of deposition and then filtered by Wattman type filter papers, No.1. centrifuge at 3000 cycles / min for 10 minutes, place the extract in glass petri dishes and leave to dry at laboratory temperature. and then collected and stored in the refrigerator at a temperature of 4 ° C.

Preparation of alcohol extract :

The extraction process was carried out by taking 20 g of plant powder used in Thumbles and then placed in the Soxhlet extractor at a temperature 45 ° C and using 400 ml of 95% ethyl alcohol for 24 hours. The rotary evaporator was then rotated at a temperature of 45 ° C. The concentrated filtrate was then left in glass petri dishes to dry at laboratory temperature,then stored in the refrigerator ^[18].

In vitro inhibition testing and determination of the minimum inhibitory concentration of plant extracts towards *Microsporumcanis*, the Agar diffusion method ^[19].was used to test the effectiveness of water, alcohol,

70.90% percentage, while the number of female infections was 32 and 29.09% percentage. The results of statistical analysis have a significant difference $P < 0.05$, $X^2 = 17.48$. The results showed that the age group 6-10 years was more likely to develop tinea capitis and 47.27%, followed by the age group 1 - 5 years and 40.00% and was the least age group to infection is the largest group of 15 years, the incidence rate was 0.90 As in Table 1, there is statistically significant difference between the age groups in the study $X^2 = 17.03834$, $P < 0.05$.

Table 1 : Percentage of age groups of people with tinea capitis

age categories	Male number	Female number	Total	Percentage
less than one year	0	2	2	1.81
1-5year	36	8	44	40.00
6-10year	38	14	52	47.27
11-15year	4	6	10	9.09
16-20year	0	1	1	0.90
21-25year	0	1	1	0.90
Total	78	32	110	100

$X^2=17.03834$, $P<0.05$

Culture Results

The most common type of fungus isolated during the study was the *Microsporum canis* (49.09%). It was found that 54 samples of the samples obtained were due to this dermatophytes. *Microsporum audouinii* and *Trichophyton verrucosum* were both isolated by 16.36%. *Microsporum gypseum* was obtained by 10.90%. *Trichophyton mentagrophytes* had a 5.45% isolation rate, and the lowest percentage was obtained for *T. violaceum* by 1.81%. As shown in Table 2.

Table (2) Total numbers and percentages of dermatophytes from persons with tinea capitis

Dermatophyte type	Number of isolates	percentage%
<i>Microsporum canis</i> Bodin	54	49.09
<i>Microsporum gypseum</i> (Bodin) Guiart and Grigorakis	12	10.90
<i>Microsporum audouinii</i> Gruby	18	16.36
<i>Trichophyton mentagrophytes</i> (Robin) Blanchard	6	5.45
<i>Trichophyton verrucosum</i> Bodin	18	16.36
<i>Trichophyton violaceum</i> Bodin	2	1.81
Total	110	100

Clinical types of Tinea capitis

During the clinical diagnosis of the samples obtained during the current study, there were two types of tinea capitis : Kerion type and Gray pach type. The samples

that showed the type of Kerion type were found to be 30 samples and 27.27% For males and 3 samples only for females, while the second type gray pach type, the number of samples 80 samples and 72.72%, the number of samples of males 54 samples, while the sample of the

females was 26 samples.

Effect of clove in *Microsporumcanis*:

The results of the present study showed a difference in the effect between the types of water extracts, alcohol, hexane, ethyl acetate, clove extract and the concentrations used. The highest effect was found for the hexane extract and at the concentration of 700 mg

/ ml. Any growth during the experiment period where the final damping results were recorded. The inhibition diameter was 8.00 cm. The results of the statistical analysis showed that there was a significant difference between the mean and the significant differences <0.05 between the types of extracts and concentrations used against *M. canis* through the values of the diameters shown in Table 3.

Table(3) Effect of Clove Extract in *Microsporumcanis*

Rates of inhibition zone (cm)						Concentration Mg / ml
Type of extract						
the average	Mixture	Ethyl acetate	Hexane	Alcoholic	Water	
5.94	4.08	5.16	8.00	7.16	5.33	700
4.72	3.16	3.66	7.16	5.5	4.16	500
3.73	1.66	3.33	5.33	4.83	3.5	200
1.22	1.35	2.25	2.53	0.00	0.00	100
0.00	0.00	0.00	0.00	0.00	0.00	50
3.12	2.05	2.88	4.60	3.49	2.59	Total
0.00	0.00	0.00	0.00	0.00	0.00	0control

L.S.D for concentrations = 0.2788

L.S.D for the abstract type = 0.2545

L.S.D for interference = 0.6234

Effect of pomegranate peel extracts in *Microsporum canis*:

The results of the pomegranate extract showed high effectiveness of *M. canis*. Both the water extract and the mixture extract had an approximately equal inhibition ratio. The inhibition diameter was 4.16 cm for the water extract and 4.00 cm for the mixture extract at the concentration of 700 mg / ml. The inhibition zone of both the extract and the ethyl acetate extract was 2.66 cm and 2.86 cm respectively , The results of the statistical analysis and the mean difference between the mean and the significant differences between $p < 0.05$ between the types of extracts and the concentrations used, as in Table 4

Table (4) Effect of pomegranate peel Extract in *Microsporum canis*

Rates of inhibition zone (cm)						Concentration Mg / ml
Type of extract						
the average	Mixture	Ethyl acetate	Hexane	Alcoholic	Water	
2.73	4.00	2.86	0.00	2.66	4.16	700
2.18	3.41	2.36	0.00	1.58	3.58	500
1.6	2.41	1.91	0.00	1.28	2.4	200
0.84	1.63	1.45	0.00	1.15	0.00	100
0.00	0.00	0.00	0.00	0.00	0.00	50

Cont... Table (4) Effect of pomegranate peel Extract in *Microsporium canis*

1.47	2.29	1.716	0.00	1.334	2.028	the average
0.00	0.00	0.00	0.00	0.00	0.00	0control

L.S.D for concentrations = 0.1185 L.S.D for the abstract type = 0.1081

L.S.D for interference = 0.2649

Effect of antifungal agents in *Microsporium canis*:

In the study of the effect of antifungal Grisovolvlin, Terbinafin and Fluconazole in *M. canis*, the highest inhibitory effect of antifungal Grisovolvlin was observed. The diameter of the inhibition zone was 7.31 cm when the concentration was 10000 µg / ml, and the diameter of the inhibition zone was 6.48 cm while the least inhibitory was found in the use of the fungal fluconazole when using the same concentration.. The results of the statistical analysis and the mean difference were significant as in table 5.

Table (5) Effect of antifungal in *Microsporium canis*

0control	the average	10000Mcg / ml	10000Mcg / ml	10000Mcg / ml	Concentration
		Rates of inhibition zone (cm)			Antifungal
0.00	6.36	5.21	6.56	7.31	Grisovolvlin
0.00	5.33	4.29	5.23	6.48	Terbinafin
0.00	0.38	0.00	0.00	1.16	Fluconazole

L.S.D for Interference concentrations = 0.1889 L.S.D for the antifungal type = 0.1636

Gas Chromatography – Mass Spectrograph (GC-MS) :

The GC-MC gas technique was used to diagnose fatty acids for the hexane extract of cloves which showed inhibitory activity against *M. canis* isolates during the study and after obtaining the curves of the compounds identified in the gas chromatograph related to the mass spectrometer. The results of the diagnosis of fatty acids

of the clove hexane extract using gas technology related to the GC-MS showed that there were 18 fatty acid species. Figure 2 show these fatty acids. The presence of Caryophyllene is very high, followed by Palmitic acid and Hexatriacontane, as shown by the presence of the following fatty acids in different levels: Myristic acid, Linoleic acid, Linolenic acid., Stearic acid.

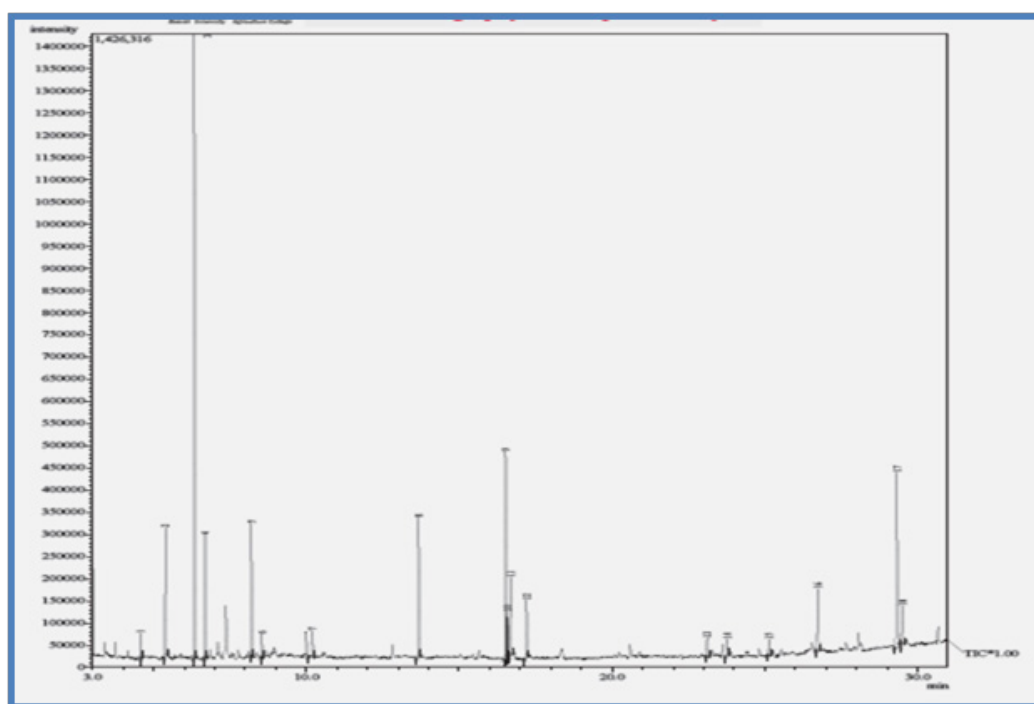


Figure (2) Fatty acids in the hexane extract of cloves using GC-MS

Conclusion

Tinea capitis is a major infection among children in Basrah city, with 61.11% of cases. The study showed that the incidence of Tinea capitis to six types of fungal fungi are: *Microsporum canis* and *M. audouinii*, *M. gypseum*, *Trichophyton verrucosum*, *T. mentagrophytes*, *T. violaceum*.

M. canis was found to be the most common type of Tinea capitis, as it was isolated from samples by 49.09%. The highest inhibitory efficacy recorded against *M. canis* was attributed to the hexane and alcohol extracts and the ethyl acetate extract of clove extracts.

Conflict of Interest : Nil

Source of Funding : Self funding

Ethical Committee Clearance : Taken

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Prevalence of Vulvovaginitis Caused by *Candida Krusei* and *Candida Glabrata* among Pregnant and Non-Pregnant Women in Tikrit City

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Abstract

Background. *Candida spp.* are considered as one of the normal flora of the genital tract in the female and can become pathogenic under some conditions that can weak the immune system or changing in the vaginal environment especially the PH of the vagina so it can cause various from Vulvovaginitis and urinary tract infection. **Aim:-** This was a cross sectional prospective study that aimed to determine the percentage of prevalence of vulvovaginitis that are caused by *candida glabrata* and *candida krusei*, and compare between pregnant and non-pregnant women. **Subject and Method:-** 200 of a high vaginal swap samples were collected from women , aged 16-55 years with symptoms of vaginal infection attending Salah-Alden General Hospital from (September 2018- January 2019). **Results:-**The results shows that *candida albicans* was the most common vaginal *Candida* species (57.1%) followed by *Candida glabrata* (25.3%) and *Candida krusei* the least percentage forming (17.4%) as a cause of vaginal candidiasis among pregnant and non-pregnant women, and the most effected age group was 36-45 years forming (54.5%) and *Candida glabrata* was more prevalent among pregnant women than *Candida krusei* while *Candida. krusei* was more prevalent among non-pregnant women.

Keywords: vulvovaginitis ; *Candida krusei* ; *Candida glabrata* ; pregnant and non-pregnant women

Introduction

Genitourinary tract infection in the female considered as one of the most serious health problem facing the community both in developing and developed countries⁽¹⁾.

Vulvovaginitis is a fungal infection of the lower female reproductive system, can be caused by many microbial agent, one of the commonest caused is the *candida* and named as vulvovaginal candidiasis or moniliosis⁽²⁾.

Candida spp. considered as one of the normal microbiotica of the lower female genital tract that form about 20%-50% in a healthy normal female⁽³⁾. The most common *candida spp.* is the *candida albicans* that also considered as the most frequent cause of vulvovaginal candidiasis ⁽⁴⁾. There is a high risk of candidal vaginal infection in pregnant women that non-pregnant women due to effect of higher estrogen levels and

higher glycogen content in secretion of vagina during pregnancy that contribute to highly risk of infection with various species of *candida* ⁽⁵⁾.

High percentage of women will experience at least a vulvovaginal candidiasis during her lifetime and the percentage may reach to 75%⁽⁶⁾.

Candidial infection of vagina is not considered as a sexually transmitted disease, because it does not affect children and it can be present normally as a normal flora, but this is not mean that if cannot transmitted by sexual contact⁽⁷⁾. *Candida albicans* account for more than 80-85% while non-*Candida albicans* account for more 5-20% of cares most species that cause vaginitis are *candida glabrata* ⁽⁴⁾ while *candida krusie* still is unusual causes but it still isolated from vaginal swabs in case of vaginitis as in this study ⁽⁸⁾.

The state of immune system contribute greatly to the incidence of vulvovaginal candidiasis so pregnancy,

using of broad spectrum antibiotic, taking of oral contraceptive pills or hormonal replacement therapy, diabetic patient and HIV. Infection all these factors increase the chance of infection with candida ⁽⁹⁾.

Candida fungi can be often isolated from urine but this is not always mean infection, this may be caused by fungi colonization of the perineum area, so isolation of candida from urine not always need for antifungal therapy ⁽¹⁰⁾. Candidial infection of urinary can be caused in special condition, especially those that are weakened the immune system such as antibiotic therapy, malnutrition, diabetics, pregnancy and catheterization ⁽¹¹⁾.

Subject and Method

This study was conducted in Gynecological department in Salah-Alden Hospital and different Gynecological clinic in Tikrit city. The study population was 200 sample of high vaginal swabs, that are collected from pregnant and non-pregnant women age between 16-55 years, the samples collected over 5 months from (September 2018 to January 2019) structured questionnaires were used to obtain data from patients, these includes age, marital state, antibiotic therapy,

main complain of the patient with clinical examination of the physicians. Then high vaginal swabs using a sterile cotton swabs from the vaginal and cervix are collected with sample of urine and each swab was inoculated separately into a Sabouraud's dextrose agar and inoculated at 25-37 C° aerobically. Thereafter, wet mounts preparation by using 10% KOH were made from the swab and examine it under light microscope for the presence of pseudohyphae and or budding yeast suggested of candida. After incubation for 24 -72 hours colonial morphology wet preparation's germ staining, germ tube test were carried out for identification of the isolated organism. Germ tube method was performed according to the bavise method was carried out to identify *candida albicans*, any negative germ tube yeast colonies were recorded as yeast rather than *candida albicans* ⁽¹²⁾. To identify other species of candida, inoculate the germ tube negative cultures into CHROM agar candida which is a selective fungal medium that includes chromogenic substances allowing for quick identification of several different *candida spp.* based on their color which also facilitates as detection of mixed infection with more than one species of candida ⁽¹³⁾. As explained in the below table 1

Table 1 show methods used for diagnosis of various *Candida spp.*

Candida species	Germ tube test	Chlamydo spores formation test	CHROMagar	Biochemical tests
				Vitek 2 System
C.albicans	+	+	Light green	98%
C.glabrata	-	-	Pinkish- purple	95%
C.krusei	-	-	Pale pink	93%

Finding

From 200 females that attending, the gynecological clinic complaining from vulvovaginitis 126 cases have a positive culture of candida. From those 126 samples 72 cases (57.1%) have infection with *Candida albicans*, 32 (25.3%) and 22 (17.4%) have infection with *Candida glabrata* and *candida krusei* respectively, as appear in the table (3) most women infected with candida krusei were in age of 36-45 years, while *candida glabrata* were

more frequently in age of 26-35 years (56.25%). This study shows also that *candida glabrata* is more frequent in pregnant ladies (65.62%) while *candida krusei* vaginal infection is more frequent in non-pregnant ladies that reaches to (54.54%) table (5). Also this study show that UTI with vaginitis is more frequent in pregnant ladies that reach to (45.9%) than non-pregnant ladies that reach to (30.8%) as appear in table (5).

Table2: frequency of *candida species* isolated from women in case study

Isolated species	Total species	
	No.	%
Candida albicans	72	57.1
Candida glabrata	32	25.3
Candida kusie	22	17.4
Total	126	

Table 3 distribution of *Candida. kusie* and *Candida. glabrata* infection according to age

Age groups	<i>C. glabrata</i>		<i>C. kusie</i>	
16-25	2	6.25	1	4.54
26-35	18	56.25	3	13.63
36-45	8	25.0	12	54.54
46-55	4	12.5	6	27.27
Total	32	100	22	100

Table4: frequency of *C. kusie* and *C. glabrata* infection in pregnant and non-pregnant women

Patients	Isolated species			
	<i>C. glabrata</i>		<i>C. kusie</i>	
	No.	%	No.	%
Pregnant	21	65.62	10	45.45
Non-pregnant	11	34.37	12	54.54
Total	32	100	22	100

Table 5: relation between vaginitis and UTI in pregnant and non-pregnant women

Patients	Vaginitis with UTI		Vaginitis without UTI	
	No.	%	No.	%
Pregnant	27	65.9	4	30.8
Non-pregnant	14	34.1	9	69.2
Total	41	100	13	100

Discussion

The female reproductive tract is considered as source of entry for number for various pathogen that are sexually or not sexually transmitted, and *candida spp.* considered as a common vaginal pathogen especially *candida albicans* that effect significantly high number

of healthy women of child bearing age⁽¹⁴⁾.

Vulvovaginal candidiasis or monilial infection of vagina is caused not just by *candida albicans* but also other species of *candida* like *Candida . glabrata* and *candida krusei* as appear by this study but in less frequency than *Candida. Albicans* and this result go

with study of Hanna Toczak⁽¹⁵⁾ Ranhul Kumar⁽¹⁶⁾, and Oamren Jactel and Katy Lai⁽¹⁷⁾, and candida positive cultures were highly in age group less than 26-45 and less frequent in age group less than 20 years and more than 40 years. This result go with the study of Akortha et al⁽¹⁸⁾ and Willacy and Jakson⁽¹⁹⁾, both of them reported peak vaginal infection between ages 20-40 years and this may be related to active sexual life in these age groups, poor personal hygiene or increased use of oral contraceptive and hormonal replacement therapy among these age group, also increased use of broad spectrum antibiotic leads to destruction of normal flora lead to decrease the vaginal immunity increasing the chance of the growth of candida infection⁽²⁰⁾.

Changing in the immune system during pregnancy, and increased hormonal loads play role in enhancing candida colonization and serve as risk factor of the vaginal infection, these effect due to dual action of both estrogen and progesterone, estrogen has been found to reduce the ability of epithelial cells of the vagina to inhibit the growth of *candida* while progesterone has been effect on the neutrophil and reduce its ability to kill *candida* spp., in this research we found that there is high frequency of *Candida glabrata* vaginitis in pregnant women reach to 65.62% while *Candida krusie* less frequent in pregnant women reach to 45.45%, and this go with the study of^(21; 20;22;23).

Candida krusie and *candida glabrata*, emerged as an important causes of vulvovaginitis in pregnant and non-pregnant women as appear in this study and many other studies⁽²⁴⁾ and it is more frequent in women more than 30 years old, and most of these patient complaining from recurrent vaginitis and this is due to highly resistant of these species of candida to antifungal drug^(25,26).

In conclusion, the large series of patient with vaginitis caused by *Candida . krusie* and *Candida . glabrata* should be considered as a cause of refractory vaginitis especially for those complaining from chronic vaginitis not responding for antimycotic drugs and an early indication for infection with a non-albicans species of *candida* may be found via routine microscopy which will reveal only blastospores on wet films.

Conflict of Interest : Nil

Source of Funding : Self

Ethical Clearance : Not required

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Toxicopathological Effect of Paraquat (PQ) and olive oil as antioxidant on Adult Albino Male Rats Epididymis

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Abstract

Paraquat (PQ) is a commonly used herbicide that induces oxidative stress via reactive oxygen species (ROS) generation. This study aimed to investigate the effects of the antioxidant (olive oil) against PQ induced oxidative stress in rats. Male rats (48) were randomly divided into 4 groups which administrated orally and daily for six weeks as follows: distilled water(C), 3mg PQ & 2ml Olive oil Kg B.wt(T1), 3mg /Kg B.wt PQ(T2) and 2ml/Kg B.wt Olive oil (T3)respectively. The animals scarified after 2,4,6 weeks to estimated the histopathological effect of paraquat and olive oil as antioxidant on adult male Epididymis of rats There was a marked vacuolar degenerated deciliation of tubules with cellular debris containing immature rounded sperms. with thickening of interstitial stroma and fibrous thinking of capsule as well as Focal infiltration of inflammatory cells and some foreign body giant cells in the lumen of tubules was seen also focal reduction in epithelial cell height other lesion showed severe distruction of epididymis structure. With tubuler atrophy & partial absent of sperms .Later increase the sperm amount inside the tubules and congested blood vessels thinking of capsule and interstitial stroma, Macrovacuolation, necrosis ,infiltration of inflammatory cells mainly lymphocytes & neutrophils in the lumen of tubules with cell debris and absent of sperms when examed at late stage of expiriment. while the olive oil group showed no clear lesions. counclusion : there Paraquat effect on male reproductive system reveled from the histopathological changes of male reproductive organ (epididymis).

Keywords: *Paraquat, olive oil, epididymis, histopathology, rats.*

Introduction

Paraquat is a quaternary nitrogen herbicide widely used for broadleaf weed control. It is a rapid acting, non-selective composite, that destroys green plant tissue on interaction and by translocation within the plant. It has been employed for killing marijuana in the U.S. and in Mexico. It is also used as a crop desiccant and defoliant, and as an aquatic herbicide⁽¹⁾. Paraquat is exceptionally lethal and causes hindrance of a few indispensable organs, (for example, the liver, kidney, and lung) and all harmed people bite the dust due to multiorgan disappointment, including significant metabolic acidosis, melancholy of myocardial or breath work because of resulting pneumonic fibrosis, and renal or hepatic disappointment⁽²⁾. A previous study suggested that free radicals which generated by PQ, are highly vulnerable to sperm membrane and mammalian epididymis, subsequently, leading to reduce sperm density⁽³⁾. Olive oil is a strong antioxidant effect, its Hydrophilic phenols are the most abundant natural antioxidants

protecting against damage from free radicals and against the formation of cancer⁽⁴⁾.

Materials and Method

Preparation of Paraquat (PQ) doses

Three mg PQ was dissolved in 100ml distilled water to prepare stock solution and prepare other doses The doses were administered daily to male rats using gastric intubation⁽⁵⁾.

Preparation of olive oil doses

The olive oil dosage was calculated by the following equation:

$$= \frac{V}{V^2} = 0.2 \text{ ml injected dose}$$

The doses were administered daily to male rats using gastric intubation (Banihani, 2017).

Experimental design

A total number of forty eight (48) male Albino Wistar rats weighting (180-220 g) were used in this experiment. Their ages ranged between (2.5-3.5) months. Experimental animals were housed in plastic cages at (22-25°C) in the animal house of department of Physiology and Pharmacology / College of Medicine -University of Maysan, with controlled lightening and the air of room was changed continuously by using ventilation vacuum. They were left for two weeks for acclimatization with the experimental conditions. Animals had free access to water and standard pellet diet along the experimental period⁽⁶⁾. Forty eight adult male rats were used in this experiment. After acclimatization for two weeks they were divided equally into four groups as follows:

Group control (C): This group received distilled water daily for 6 weeks

Group one (T1): This group received (3mg & 2ml/ Kg B.wt) PQ & Olive oil daily for 6 weeks.

Group two (T2): This group received (3mg/Kg B.wt) PQ only daily for 6 weeks

Control three Four (T3): This group received (2ml/ Kg B.wt) Olive oil only daily for 6 weeks..

The experiment was lasted for 6 weeks. after 2, 4, 6 weeks of the experiment, three animals from each group were anesthetized and killed for the histological study of epididymis.

Results

Histopathological examination

T1 group(PQ & Olive oil)

The epididymal changes demonstrating epithelial with no clear lesion, accumulation of sperm in the tubules lumen (fig-1) also absorbed at 4th weeks after administration epithelium vacuolar degenerated deciliation of tubules with cellular debris containing immature rounded sperms(fig-2).while after 6th weeks showed thickening of interstitial stroma and fibrous thinking of capsule as well as Focal infiltration of inflammatory cells(fig-3).

T2 group (Olive oil)

The section of epididymis showed epithelial cells vacuolation as well as infiltration of inflammatory cells mainly foreign body giant cells in the lumen of tubules with focal reduction in epithelial cell height. w4 showed severe distortion of epididymus structure. With tubular atrophy & partial absent of sperm showed Macrovacuolation, necrosis, infiltration of inflammatory cells mainly lymphocytes & neutrophils in the lumen of tubules with cell debris and absent of sperms

T3 group(PQ)

of normal rat epididymis w6 showed increase the sperm amount inside the tubules and congested blood vessels thinking of capsule and interstitial stroma.

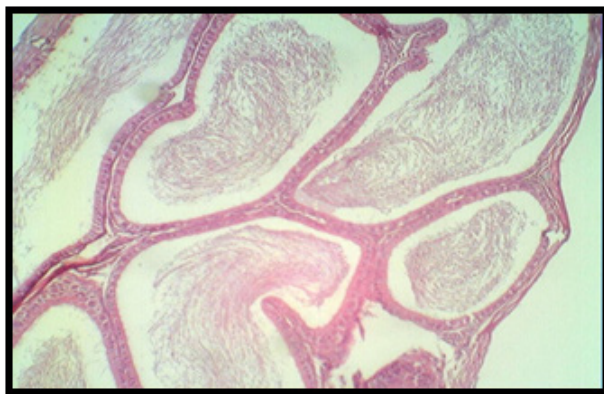


Figure-1 :Histopathologic section of rat epididymis(T1w2) that showed no clear lesion accumulation of sperm in the tubules lumen. (H&E stain 100X)

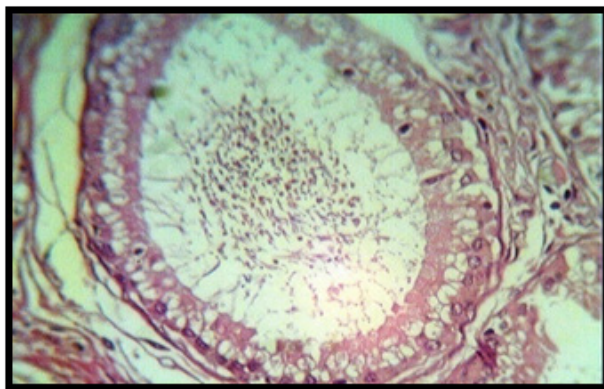


Figure-2 :Histopathologic section of rat epididymis (T1w4) group showed with epithelium vacuolar degenerated deciliation of tubules with cellular debris containing immature rounded sperms(H & E stain, 400X).

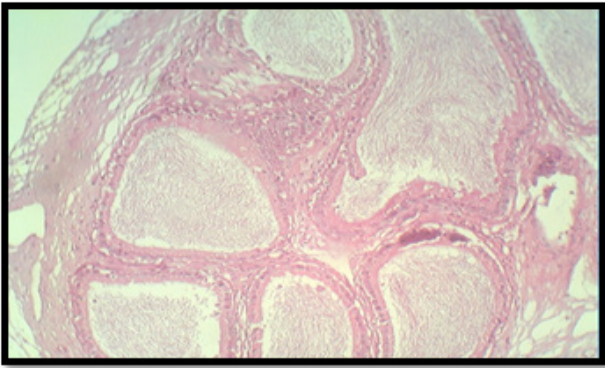


Figure-3 : Histopathologic section of rat Epididymis (T1W6) showed thickening of interstitial stroma and fibrous thickening of capsule. Focal infiltration of inflammatory cells (H&E stain, 100X).

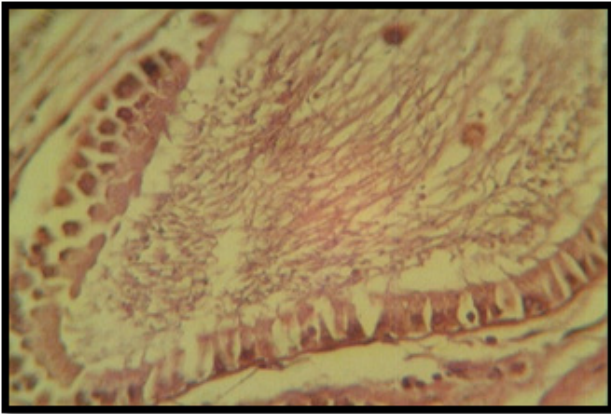


Figure-4 : Histopathologic section of rat epididymis (T2W2), showed epithelial cells vacuolation, infiltration of inflammatory cells mainly foreign body giant cells in the lumen of tubules, focal reduction in epithelial cell height. (H&E stain, 400X).

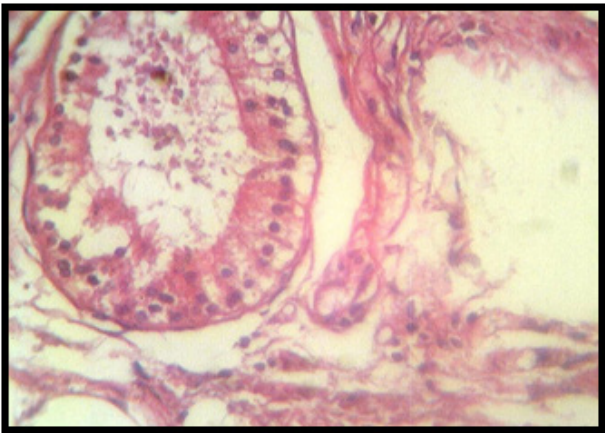


Figure-5: Histopathologic section of rat epididymis (T2W4), showed Macrovacuolation, necrosis, infiltration of inflammatory cells mainly lymphocytes & neutrophils in the lumen of tubules with cell debris and absent of sperms (H&E stain, 400X).

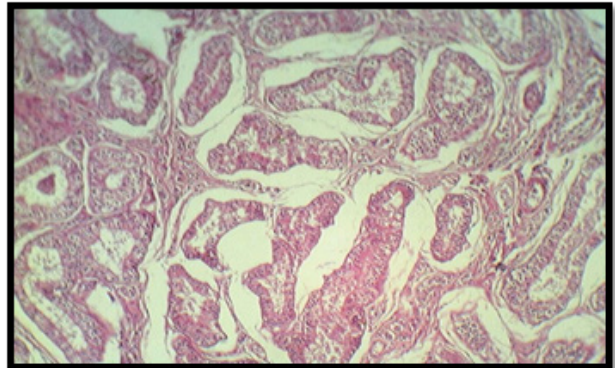


Figure- 6: Histopathologic section of rat epididymis (T2W6), showed severe distortion of epididymus structure. With tubular atrophy & partial absent (H&E stain, 100X)

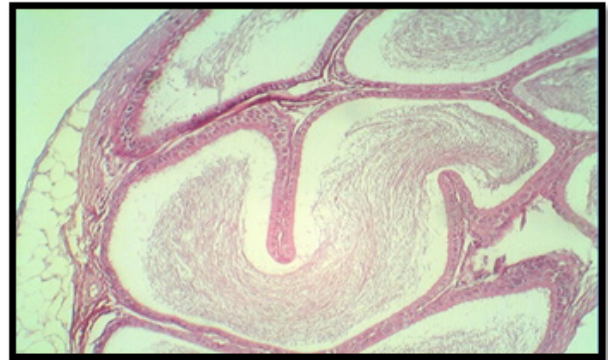


Figure-7 : Histopathologic section of normal rat epididymis (T3w4) (H&E stain, 100X).

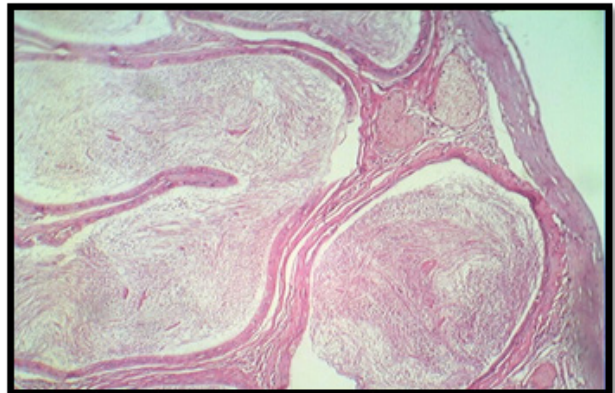


Figure-8 : Histopathologic section of rat epididymis (T3W6), showed increase in sperm amount inside the tubules and congested blood vessels thickening of capsule and interstitial stroma. (H&E stain, 100X).

Dissection

It is well known that sperm is highly susceptible to reactive oxygen species⁽⁷⁾. Because reactive oxygen species will harm lipid layers of sperm thus sperm membrane is in danger. As Paraquat produces free radicals⁽⁸⁾, which can damage sperm membranes. similar mechanism has happened in the present investigation while might have been a probable case for reduction in sperm motility as⁽⁹⁾

reported. The destruction process has an involvement with epididymal epithelium. But as we know that Paraquat can generate free radicals within the biological system. These free radicals are highly vulnerable to membrane structures including sperm membrane. Per oxidation and damages to the membrane will alter all the functions of the membrane creating a hostile situation to sperm function in the epididymis, that approve with ⁽¹⁰⁾. The dietary supplementation of extra virgin olive oil counteracted the damage effect of the pesticide by the improvement of antioxidant defence system and the drop of the lipid peroxidation. ⁽¹¹⁾. Olive oil ameliorates adverse effects of oxidative damage on reproductive organs in adult male albino rats. This protective action of olive oil effects which may protect or restore fertility justifies its use against the oxidative damage induced in reproductive organs⁽¹²⁾.

Conclousion

Paraquat effect on male reproductive system revealed from histopathological changes of epididymis while the daily consumption of Olive oil can decrease stress oxidative and alteration spermatogenesis in albino rats.

Conflict of Interest :None

Source of Funding :Self

Ethical Clearance: All samples taken after the patients' approval.

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Nephrotoxic Effect of Antipsychotic Drug Risperidone : Protective Role Extract of *Urtica dioica* leaf in Male Stressed Rats

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Abstract

This research was designed to investigate the ameliorative effect of *Urtica dioica* leaf extract on the kidney function against Risperidone . Thirty adult male rats were randomly divided to 3 equal group and handles as follows for 4th weeks: control group were administered tap water group G1 ; group G2 rats of this group had been administered risperidone **2 mg/kg orally only**; group G3 rats of this group had been administered risperidone **2 mg/kg orally** plus ethanolic extract of *Urtica dioica* (100 mg/kg) . Blood sample were obtained at 15 and 30 days of the experiments for estimation of serum uric acid concentration , serum urea nitrogen concentration, serum creatinine concentration , serum peroxynitrite concentration. Significant increases in estimated parameters in group G2 (risperidone treated group) . On the other hand, the results showed the beneficial effects of *Urtica dioica* leaf extract to improvement the previous parameters against risperidone (G3) , through a significant decrease in concentrations. Histological section show atrophy and acute necrosis in renal tubules in group G2, while no clear pathological lesion in G3 group . Therefore, it could be conclude that *Urtica dioica* exert a protective action to alleviating the oxidative stress induced by risperidone stressed rats.

Keyword: Nephrotoxic, Risperidone, *Urtica dioica* , rat .

Introduction

Risperidone, derivative of a benzisoxazole, is a familier of the most orderly specified antipsychotics in the management of autism , schizophrenia and demeanor disorders ^[1] . Even- thought the evaluate of exceptional antipsychotic drugs have been effective in the assistance of demeanor disorders and schizophrenia, there could aggravate some complications, including drowsiness, sedation, sight difficulties, weight get ,constipation, hyperprolactinemia , activity problems and another side- effects ^[2] . One of critical organs maintaining homeostasis of body is the kidney and for that reason any disorder of kidney impact the quality of life and health ^[3] . Risperidone and its essential active metabolite -9hydroxyrisperidone , were rejected through the kidneys. In patients with renal disease, clearance of the sum of the origin drug and metabolite have been demonstrating to reduce in 60% compared to healthy subjects^[4] . Few cases notify atypical antipsychotic drug-associated renal damage in patients (including

those using RIS). Pernicious outcomes potentially attributable to those drugs, such as decrease blood pressure, acute urinary retention, and rhabdomyolysis , are known to cause its injury ^[5] . Risperidone prompt oxidative stress through inducement of ROS such as superoxide, hydroxyl radical anion and hydrogen peroxide, that attacked different cell components as DNA, RNA, proteins, lipids and enzymes leading to many degenerative processes in the renal cells manifested as glomerular disorders , renal ischemia, perfusion injury and finally acute renal failure ^[6].

Urtica dioica was commonly known as “Stinging Nettle”,having a place with the family Urticaceae ^[7]. It rich in vitamins such as A, thiamine (B1), riboflavin (B2), C, D, E, K, and is loaded with minerals such as calcium, cobalt, magnesium, chromium, phosphorus, copper, iron, potassium, silicon, sulfur and zinc various phytochemicals and their effect on suppression of active oxygen species by natural antioxidants have been intensively studied^[7,8]. In Germany, Nettle leaf

has extended history of traditional medicinal use for arthritis. *Urtica dioica* herb has been utilized for different diseases including genitourinary ailments (nocturia, frequency, dysuria, urinary retention, irritable bladder, and infections), kidney dysfunction, allergies, diabetes, internal bleeding (including uterine bleeding, epistaxis, and melena), anemia, GI tract ailments (diarrhea and dysentery, and gastric hyperacidity), musculoskeletal aches and alopecia^[9,10]. Therefore, the current work was designed to investigate the potential beneficial effect of *Urtica dioica* against nephrotoxicity induced by Risperidone in rats.

Material and Method

The leaves of *Urtica dioica* were taken from north of Iraq. To prepare 70% ethyl alcohol, 100 grams of dried leaves which was mixed with 500 ml of alcohol in each extract process by using magnetic stirrer at 40 °C for 24 hours^[11, 12], the extract have been filtrated and the process have been repeated 3 times. The filtrate was concentrated by using incubator at 40 C° for 72 hr to obtained crude plant extract, the result kept at 4 C° in sterile and dark glass container until used.

Thirty adult male rats were randomly divided to 3 equal group and handles as follows for 4th weeks :group G1 control group were administered tap water ; group G2 rats of this group had been administered risperidone **2 mg/kg orally only** ; group G3 rats of this group had been administered risperidone **2 mg/kg orally** plus ethanolic extract of *Urtica dioica* (100 mg/kg), collected of blood sample were obtained via cardiac puncture from each anesthetized animal at 15 and 30 days of the experiments then centrifuged at 3000 rpm for 15 minutes, and sera was isolated and frozen at -18C° till analysis of serum uric acid concentration, serum urea nitrogen concentration, serum creatinine concentration, serum peroxynitrite concentration. Furthermore, At the end of experiment kidney were excised and fixed in 10% formalin buffer solution for histological examination. Tissue sections had been prepared and stained with Hematoxylin-Eosin stain (H and E) according to^[13]. Two way analysis of variance (ANOVA) statistical analysis of data was perform within this basis, using a significant level at (P<0.05) and using Least Significant Differences (LSD) test for specific group differences^[14].

Results and Discussion

After 15 to 30 days of treatment with risperidone

group(G2) recorded a significantly (P<0.05) increment in serum uric acid concentration as compared to control and (G3) group (table 1). On the other hand, male rats administered risperidone concurrently with *Urtica dioica* extract group (G3) caused a significantly (P<0.05) decrement in this parameter in 15 and 30 days of treatments periods comparing to group(G2). Moreover, the value of serum uric acid concentration tended to increase significantly (P<0.05) after 30 days of experiments in (G2) as compared to 15 days. In contrast, treatment of rats *Urtica dioica* extract combined with risperidone showed significantly(P<0.05)decrement in serum uric acid concentration in 30 days compared 15 days of treatment.

Serum blood urea nitrogen concentration significantly (P<0.05) increase in Risperidone treated group at 15 and 30 days of experiments comparing to *Urtica dioica* extract treated group concurrently with Risperidone G3 and control group (table 2) . At end of experiment highest significant (P<0.05) reduction in serum blood urea nitrogen concentration were observed after administered *Urtica dioica* extract in G3 group comparing to G2 group.

Within groups, significant (P<0.05) elevation in serum blood urea nitrogen concentration in G2 treated group were observed at end of experiments comparing to 15 days of treatment period.

Depending on the results clarified in table (3), there was a significantly (P<0.05) increment in risperidone treated rats group G2 in serum creatinine concentration as compared to experimental groups G1 and G3 after 15 and 30 days of experiments . On the other hand , oral intubation *Urtica dioica* extract in combination with risperidone caused a significant (P<0.05) decrease in this parameter after 15 and 30 days with mean value (1.22±0.01) (1.01±0.05) as compared to risperidone treated group (1.82±0.04) (2.24±0.02) respectively. Besides, the statistical analysis recorded a significant (P<0.05) gradual increase of serum creatinine concentration in G2 group, whereas group G3 registered a significant decrease in this parameter . While control group recorded non-significant(P>0.05) differences at 15 and 30 days of experiments when compared with each-others.

Data pertaining, in table (4) recorded a significantly(p<0.05)increment in serum peroxynitrite

concentration in adult male rats, in group G2 as compared to experimental groups in two treated periods . On the other hand the results showed that intubation rats *Urtica dioica* extract group G3 ameliorating the effect of risperidone on serum peroxynitrite concentration and result recorded a significant ($p<0.05$) decrease in this parameter after 15 and 30 days of experiments as compared to group G2 , indicating the antioxidant effect of *Urtica dioica* extract against Risperidone . In comparison within the time (15 and 30 days) for G2 and

G3 groups, the concentration serum peroxynitrite of group G2 recorded a significant ($p<0.05$) increase, whereas , G3 group registered a significant gradual decrease in this parameter. On the other hand , histological section of kidney rats received RIS show atrophy renal tubules(figure 2) and acute tubular necrosis(figure 3), while in rats received *Urtica dioica* extract concurrently with RIS show no clear pathological lesion (figure 4) and no abnormality when compared to control group (figure 1) .

Table (1) serum uric acid concentration (mg/dl) in rats administered risperidone and *Urtica dioica* extract

mean \pm SE/ n=10 each		
Group	15 day	30 day
G1	3.15 \pm 0.01 C a	3.15 \pm 0.01 C a
G2	4.78 \pm 0.02 A b	5.53 \pm 0.01 A a
G3	4.16 \pm 0.01 B a	3.34 \pm 0.01 B b

LSD 0.05 G1:control; G2: administration risperidone 2 mg/kg orally only; G3: administration risperidone 2 mg/kg orally plus ethanolic extract of *Urtica dioica* (100 mg/kg); capital letters denotes differences between groups , $p<0.05$ and small letters denotes significant differences within group ($p<0.05$)

Table (2)serum blood urea nitrogen concentration (mg/dl)in rats administered risperidone and *Urtica dioica* extract

mean \pm SE/ n=10 each		
Group	15 day	30 day
G1	17.45 \pm 0.15 C a	17.39 \pm 0.16 C a
G2	31.05 \pm 0.31 B b	55.54 \pm 0.91 A a
G3	25.01 \pm 0.63 A a	19.24 \pm 0.30 B b

LSD 1.4

Note for details show table 1

Table (3) Serum creatinine concentration (mg/dl) in rats administered risperidone and *Urtica dioica* extract

mean \pm SE/ n=10 each		
Group	15 day	30 day
G1	0.71 \pm 0.005 C a	0.71 \pm 0.004 C a
G2	1.82 \pm 0.04 A b	2.24 \pm 0.02 A a
G3	1.22 \pm 0.01 B a	1.01 \pm 0.05 B b

LSD 0.09

Note for details show table 1

Table (4) serum peroxynitrite concentration (μ mol/L) in rats administered risperidone and *Urtica dioica* extract

mean \pm SE / n=10 each		
Group	15 day	30 day
G1	32.55 \pm 0.21 C a	32.55 \pm 0.21 C a
G2	43.03 \pm 0.19 A b	46.90 \pm 0.30 A a
G3	37.26 \pm 0.05 B a	33.52 \pm 0.52 B b

LSD 0.6

Note for details show table 1

Discussion

Exclusive detrimental consequences attributed to abnormal antipsychotic drugs are regarded to cause renal dysfunction, this research detected the possible toxicity of the risperidone on the kidney. Serum uric acid, Cr and BUN have been the classical standards to assess renal damage [15]. RIS might impair tubular function and give rise to several renal complications. The results of this study demonstrate that RIS exposure induced a significant increase in the level of serum Cr, BUN and uric acid indicate disrupt structural and functional kidney entirety.

The creatinine is a waste product excreted by the kidney mainly through glomerular filtration. When the value of this product increase this indicates decreased excretion or impaired renal function [16]. Creatinine clearance can a estimation of the glomerular filtration rate. The nephrotoxic effects may be also because the direct action of risperidone on renal mitochondria, the kidney includes further mitochondria in comparison to different organs, the reduction consequences of those compounds on mitochondrial function may be essential in the pathogenesis of nephrotoxicity [17].

One liver protein derived from diet or tissue source that is blood urea nitrogen and is normally excreted in the urine [18], this test was been indicated of deteriorate renal function that one of the reliable tests of a renal

disorder.

Risperidone treated group showed a significant increase serum Cr, uric acid and BUN during the treatment period were in accordance with nephrotoxic effect caused by risperidone. While those groups treated with *Urtica dioica* plus risperidone restoration of the elevated serum Cr, uric acid and BUN to the normal levels. Consistent with those observations, the useful effects of *Urtica dioica* were diagnosed in the prevention of renal tubular damage and disorders. The results of tests can suppose the nephroprotective effect shown by *Urtica dioica* in risperidone-induced nephrotoxicity [19].

In the present study, administration of Risperidone to rats resulted in development of oxidative stress showed by significant increase in serum peroxynitrate concentration, also induced nephrotoxicity showed a significant ($P < 0.05$) increase in the serum creatinine, uric acid and blood urea nitrogen concentration in G2 treated group compared to other treatment groups display decrease in same parameter. Generation of reactive oxygen species (ROS) contribute to cellular damage [8], evidence to indicate that oxidative stress can be the more serious component within the pathophysiology and effects of toxicities concerning to antipsychotic medication [20]. Oxidative stress have a critical role in the pathophysiology of numerous kidney diseases, and many complications of these diseases are mediated via oxidative stress, oxidative stress-associated mediators and inflammation [21,22]. The kidney is an organ fairly vulnerable to deterioration as a result of ROS, possible due to the abundance of long-chain polyunsaturated fatty acids at the composition of renal lipids [23].

The study indicated histopathological changes in kidney rats treated with Risperidone. Risperidone can negatively impacts the kidney through atrophy of renal tubules and indicates acute necrosis. The consequences concerning the impact of risperidone in the current study is in accordance with other research [24]. It was supposed that a correlation between the histological changes and oxidative stress induced by risperidone in this study. So oxidative stress attributed to finding of this research with a serious damage in kidney tissue [8, 24].

Previous histological study from kidney section reflected the protective role of nettle in preventing the appearance of renal abnormal changes [25, 26]. The protective activity of *Urtica dioica* might due to

phenols. Phenolic compounds contain antioxidant properties due to ability of scavenging free radicals and active oxygen species such as single oxygen, free radicals and hydroxyl radicals [27,28]. In **conclusion**, the component of *Urtica dioica* extract have a potential role in preventive of renal dysfunction from detrimental effect of antipsychotics drug risperidone.

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Conflict of Interest: There is no conflict of interest.

Source of Funding :Self

Ethical Clearance: Non

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Molecular Marking of Liver DNA Methylation in Rat's with Thyroid Carcinoma

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Abstract

Altered expressions of microRNAs also silence or activate many genes in progression. Methylation can change the activity of a DNA segment without changing the sequence. Western blot analyses explained an activation for a mitogen-activated protein kinase (MAPK or MAP kinase) is a type of protein kinase that is specific to the amino acids serine and threonine.

Keywords : *Molecular marking ; DNA methylation ; thyroid carcinoma*

Introduction

Thyroid cancer is cancer that develops from the tissues of the thyroid gland. It is a disease in which cells grow abnormally and have the potential to spread to other parts of the body.⁽¹⁾

Thyroid cancers can be classified according to their histopathological characteristics into four main types – papillary thyroid cancer, follicular thyroid cancer, medullary thyroid cancer, and anaplastic thyroid cancer.⁽²⁾

DNA methylation is a process by which methyl groups are added to the DNA molecule. Methylation can change the activity of a DNA segment without changing the sequence. When located in a gene promoter, DNA methylation typically acts to repress gene transcription. DNA methylation is essential for normal development and is associated with a number of key processes including genomic imprinting, X-chromosome inactivation, repression of transposable elements, aging and carcinogenesis.^(3,4)

Aim of this study is to investigate the disorder in methylation of rat liver's DNA in thyroid carcinoma cases.

Materials and Method

1- Site of study: at the laboratories of al-forat al-Awsat university.

2- Histopathology sections: collection of tissue samples, chemical fixation, processing stages and staining with hematoxylin-eosin stains applied according to John D. Bancroft et al.⁽⁵⁾

3- Genetic analysis: including application of western blot analysis according to Li Fan et al.⁽⁶⁾

4- Statistical analysis: all the inputs and issues tabulated and scheduled by computerized statistical programme (SPSS) established on the interact articles to get fine assessment. Julien I.E. Hoffman (2015).⁽⁷⁾

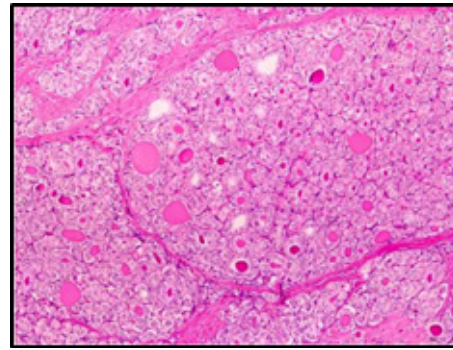
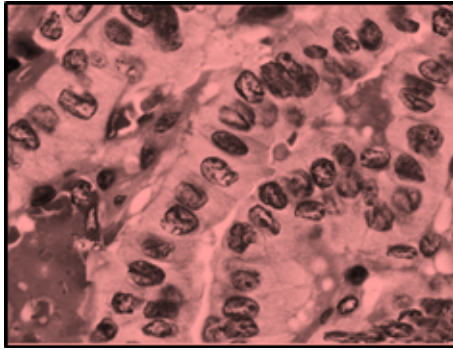
Results

Papillae among its cells, visible on microscopy. Features include characteristic Orphan Annie eye nuclear inclusions (nuclei with uniform staining, which appear empty due to powdery chromatin and marginal micronucleoli) and psammoma bodies on light microscopy. The former is useful in identifying the follicular variant of papillary thyroid carcinomas. Lymphatic spread is more common than hematogenous spread. Multifocality is common, the so-called Lateral Aberrant Thyroid is actually a lymph node metastasis from papillary thyroid carcinoma.

Papillary microcarcinoma is a subset of papillary

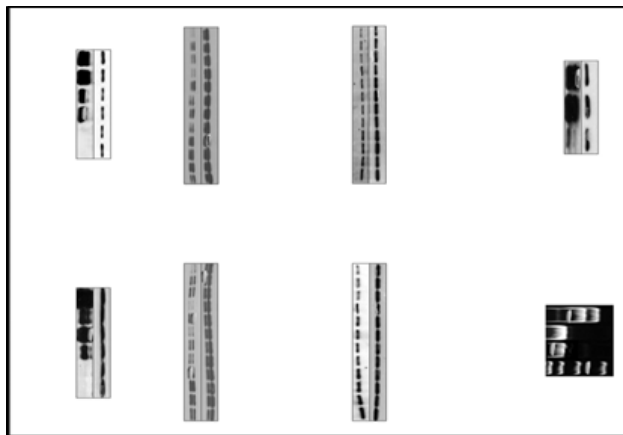
thyroid cancer defined as measuring less than or equal to 1 cm. The highest incidence of papillary thyroid microcarcinoma in an autopsy series was reported by Harach et al. in 1985, who found 36 of 101 consecutive autopsies to have an incidental microcarcinoma. Michael Pakdaman et al. report the highest incidence

in a retrospective surgical series at 49.9 percent of 860 cases. Management strategies for incidental papillary microcarcinoma on ultrasound (and confirmed on FNAB) range from total thyroidectomy with radioactive iodine ablation to observation alone.



Papillary microcarcinoma is a subset of papillary thyroid nuclei with uniform staining, which appear empty due to cancer defined as measuring less than or equal to 1 cm powdery chromatin and marginal micronucleoli

Western blot analyses explained an activation for A mitogen-activated protein kinase (MAPK or MAP kinase) is a type of protein kinase that is specific to the amino acids serine and threonine (i.e., a serine/threonine-specific protein kinase). Another activation was being obvious for rearranged during transfection kinase and B-Rapidly Accelerated Fibrosarcoma gene kinase. Look below.



MAPK RET/PTC BRAF Western blot analyses explained an activation for MAPK, RET, PTC and BRAF

Discussion

In cancer, gene promoter CpG islands acquire abnormal hypermethylation, which results in transcriptional silencing that can be inherited by daughter cells following cell division. Alterations of DNA methylation have been recognized as

an important component of cancer development. Hypomethylation, in general, arises earlier and is linked to chromosomal instability and loss of imprinting, whereas hypermethylation is associated with promoters and can arise secondary to gene (oncogene suppressor) silencing, but might be a target for epigenetic therapy. (8,9)

Global hypomethylation has also been implicated in the development and progression of cancer through different mechanisms. Typically, there is hypermethylation of tumor suppressor genes and hypomethylation of oncogenes. (10)

Generally, in progression to cancer, hundreds of genes are silenced or activated. Although silencing of some genes in cancers occurs by mutation, a large proportion of carcinogenic gene silencing is a result of altered DNA methylation. DNA methylation causing silencing in cancer typically occurs at multiple CpG sites in the CpG islands that are present in the promoters of protein coding genes. (11,12)

Altered expressions of microRNAs also silence or activate many genes in progression to cancer. Altered microRNA expression occurs through hyper/hypomethylation of CpG sites in CpG islands in promoters controlling transcription of the microRNAs. (13)

Silencing of DNA repair genes through methylation of CpG islands in their promoters appears to be especially important in progression to cancer.

Conflict of Interest : Nil

Source of Funding : Self funding

Ethical Committee Clearance : Non

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Assessment Infectious Incident for Mumps Epidemic among Iraqi Population in Statistics Center of the Iraqi Ministry of Health

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Abstract

Mumps is an infection caused by a virus. It is sometimes called infectious parotitis, and it primarily affects the salivary glands. The aims of this study to assess the prevalence of mumps cases according to some demographic characteristic during the study period in Iraq. Retrospective cross-sectional study was conducted at the Statistics Center of the Iraqi Ministry of Health to collect the data samples. Samples were included age, region, gender, and years. Samples were collected within six months after obtaining the ethical clearance from the Ministry of Health before conducting the research. data entered to Excel sheet and then analysis by Stata version 13. The table and graphs were used to present our data. Higher frequency of infection occurred in the age groups less than 15 years. Half of infection 56.9% (73939/129971) were occurred in 2016 and less 1% (1295/129971) in 2014. The incidence rate of infection among people per 10000 of the were 0.58%, 0.49%, 0.52%, 0.36%, 3.5%, 19.5% and 9.79% respectively. Male cases of infection were more than female. the higher percentage of infection was occurred during 2016. Try to educate people about the risk of mumps through seminars.

Keyword: - Epidemiology, Mumps, Iraq, Male, Years, Infection

Introduction

Mumps is an infection caused by a virus. It is sometimes called infectious parotitis, and it primarily affects the salivary glands⁽¹⁾. Mumps is mostly a mild childhood disease⁽²⁾. It most often affects children between five and nine years old. But the mumps virus can infect adults as well. When it does, complications are more likely to be serious⁽¹⁾. Mumps virus is present throughout the world. It is spread by airborne droplets released when an infected person sneezes or coughs and by direct contact with an infected person⁽³⁾. In Iraq, the incidence rate of infection is varying among people per 10000 of the population during the seven years (4-10). According to several studies resurgence of mumps epidemics has been documented by many investigators during this decade in several countries⁽¹¹⁾. In addition to other countries in Asia, Africa, Middle East and in north America⁽¹²⁻²¹⁾. This contagious disease is widely distributed in low- and medium-

income countries like Iraq, mumps is an insignificant and neglected public health problem because of poor documentation of clinical cases and lack of published epidemiologic studies⁽²²⁾. Studies have confirmed the cost-effectiveness of prevention and control of mumps through the reduction of school- and work-absenteeism and reduction in costs of treatment of complicated cases and the associated hospitalization^(23,24). This study has been conducted to assess the prevalence of mumps cases according to some demographic characteristic during the study period in Iraq.

Method

Retrospective cross-sectional study was conducted at the Statistics Center of the Iraqi Ministry of Health to collect the data samples. Samples were included age, residence, gender, and years. Samples were collected within six months after obtaining the ethical clearance from the Ministry of Health before conducting the

research. data entered to Excel sheet and then analysis by Stata version 13. The table and graphs were used to present our data.

Findings

One hundred and twenty-nine thousand and nine hundred and seventy-one of cases had mumps were recruited in this study for both male and female with different age groups, in 2011, the total number of cases were 1930 (1.5%). The higher frequency of infection 1678 in the age groups less than 15 years followed by 192 in the age groups 15-45 years and less frequency 60 in the age more than 45 years. Also, during the 2012, the total cases of mumps were 1674. the higher frequency 1482 in the age less than 15 years and less frequency 9 in the age >45 years. In addition, in 2013, 2014,

2015, 2016 and 2017, the higher frequency 1663, 1001, 8724, 54384 and 23675 in the age groups less than 15 years, respectively [Table 1]. According to age group, the current study showed that the higher percentage 71.3% in the age groups less than 15 years old was exposed to infection more than other age groups. This result agreement with two studies done in Tehran by ⁽²⁵⁾ they found that children between 0-9 or 5-9 years old had highest susceptibility to mumps infection. Another study done conducted in USA found that the susceptibility to mumps infection arrived to 90% in children under 14 years old ⁽²⁶⁾. Several factor may be responsible for this outbreak and the most important one immunogenicity, efficacy, and safety of mumps vaccine it was used as well as storage condition.

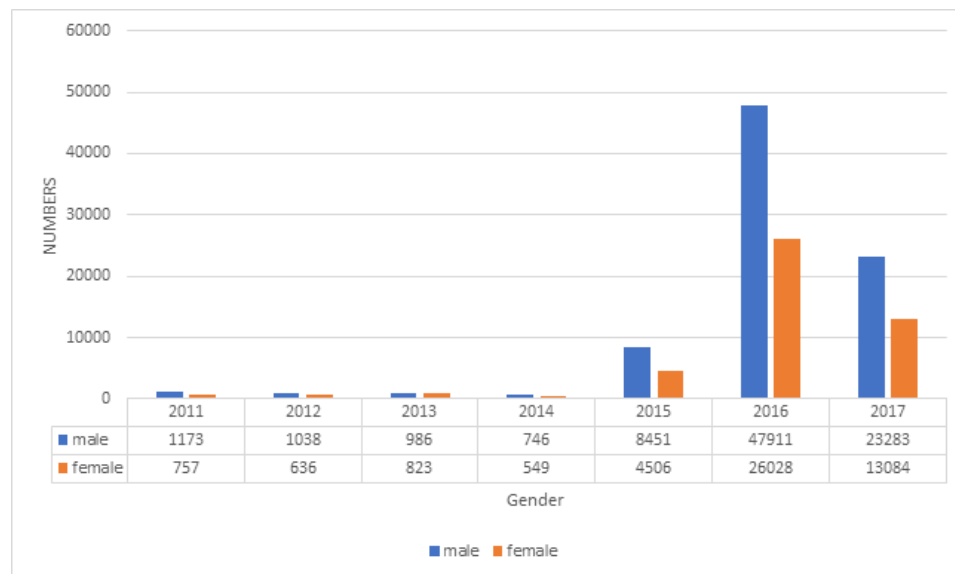


Figure 1: Distribution of Mump cases according to gender by years in Iraq

Table 1: - Distribution of cases according to age groups by years in IRAQ.

Age groups (years)	Years							Total
	2011	2012	2013	2014	2015	2016	2017	
	F %	F %	F %	F %	F %	F %	F %	
<15	1678 1.8%	1482 1.6%	1663 1.8%	1001 1.1%	8724 9.4%	54384 58.7%	23675 25.6%	92647 100%
15-45	192 0.5%	143 0.4%	136 0.4%	214 0.6%	3813 10.8%	18562 52.7%	12168 34.5%	35228 100%
>45	60 2.9%	9 0.4%	10 0.5%	80 3.8%	420 20.0%	993 47.4%	524 25.0%	2096 100%
Total	1930 1.5%	1674 1.3%	1809 1.4%	1295 1.0%	12957 9.9%	73939 56.9%	36367 27.9%	129971 100%

Regarding to gender and years, the higher frequency of infection among male more than female cases [Figure 1].Also, the higher percentage of infection was reported 64.3% among male cases more than female cases 35.7% during the study period. This result was compared with another studied (3)(27-29). And may be related with high risk activities in males as compared with females such as occupational factors, travel and social differences.

Concern to distribution of total cases of mump by years. the higher frequency of infection 56.9% (73939/129971) was occurred in 2016 and less frequency of infection 1% (1295/129971) in 2014[Figure 2].

The incidence rate of infection among people per 10000 of the population during 2011, 2012, 2013, 2014, 2015, 2016, and 2017. 0.58%, 0.49%, 0.52%, 0.36%, 3.5%, 19.5% and 9.79% respectively [Figure 3]. The high rates of mumps infection among Iraqi cites in the current study this may be related with the high susceptibility of the target groups, low level of public awareness about the transmission of the disease and the dropping lets precautions, overcrowding, un healthy housing and improper behaviors of patients and their families with the disease during epidemics such as the social un healthy habit of kissing which is very common in our society(5-10).

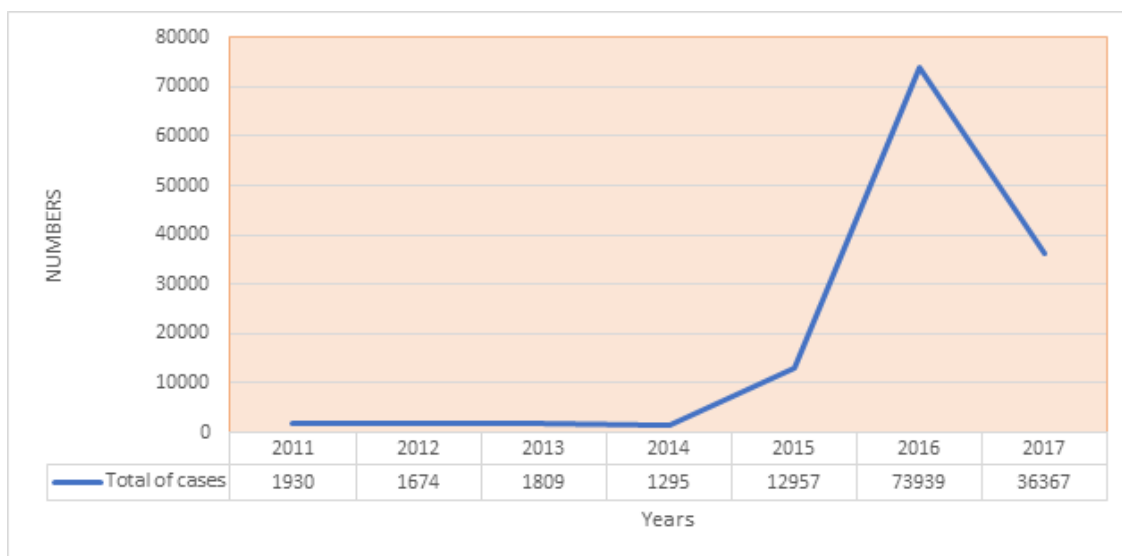


Figure 3: Incidence rate of infection per 10000 of population during the study period in Iraq

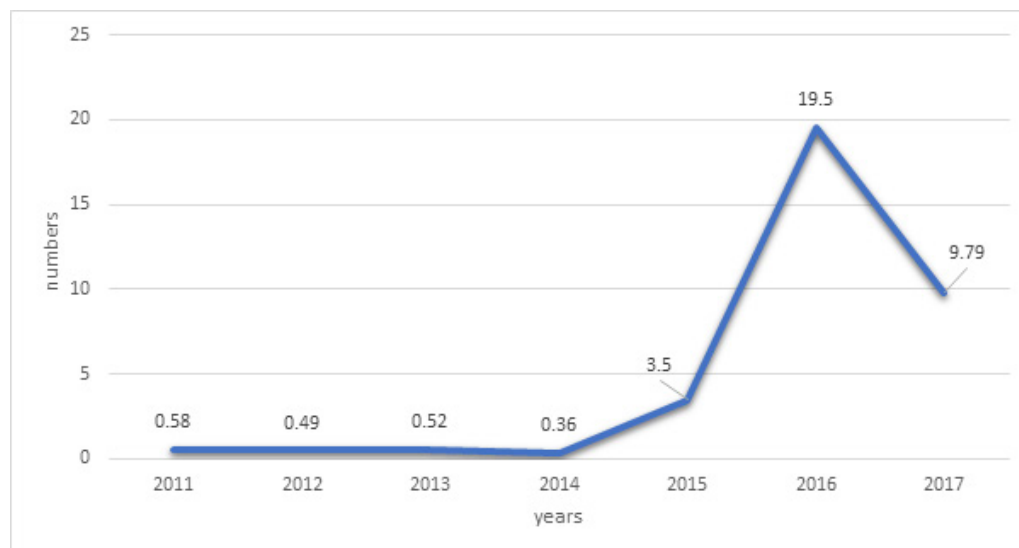


Figure 3: Incidence rate of infection per 10000 of population during the study period in Iraq

Conclusions

The male cases of infection were more than female. the higher percentage of infection was occurred during 2016. Try to educate people about the risk of mumps through seminars.

Ethical Clearance: the permission was obtained from the Statistics Center of the Iraqi Ministry of Health before conducting the study.

Conflict of Interest: Nil

Source of Funding: Nil

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Isolating and Diagnosis Some Genus of Fungi that Found in Environment Air and Soil in Different Region of Wassit City

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Abstract

This study was carried out in the second half of April 2018 in waist governorate in Iraq to isolate some fungi that are important in their human health or its economic side. It was isolated and diagnosed from the soil and air from various regions in wasit governorate include the regions:/kut technical institute, kafaat, tammoz, damok, nu'maniyah, deboni and aziziyah. This study included four sites for each region and four replicates for each site, using 112 cultivars for the growth and diagnose of fungi in the regions and sites studied. These genus of fungi were identified by depend on based "phenotypic ,microscopicand the growth of fungi on media culture PDA and SDA.The results showed that the most common fungal species in the air were Aspergillus, which showed that the higheremergence percentage reaches 91.57% and theemergence percentage of the fungi,Penicillium, Rhizopus, Mucor and Ustilago was 77.57, 11.14, 8 and 7.85%, respectively. The most commonemergence percentage of the fungi species in the soil environment were Fusarium, Cladosporium, Pythium, Pytophthora,RhizoctoniaMicrosporum, Aspergillus and Penicillium. The percentage of the incidence of these fungi was 34, 19.28, 19, 15.42, 12.85,10.71,7.85and 6.42% in the regions and sites were studied.

Keywords: *Fungi in air environment, Fungi in soil environment,Funi in wassit environment.*

Introduction

Fungi are eukaryotic organisms that do not contain chlorophyll, they are heterotrophic organisms, so they either live on the dead residues of both plant and animal organisms (saprophytic fungi) or live parasitic on animal hosts (parasitic fungi) which are widespread and commonly found in all areas where moisture and heat are available. They are found in soil, air, fresh water and saline. Some single-cell fungi consist of only one cell that performs all vital functions such as yeast, but most of them are multicellular organisms that are composed of several regular cells and grow as branching hyphae. These are formed in the form of mycelium, which can be divided by walls or septum. Or don't divided "coenocytic".

The fungi reproduce in a variety of ways: budding, fragmentation, clamidiospores,conidia,Fungi can be isolated from the soil, air and water. Where organic matter is found, the quality of the isolated fungi varies depending on the type of soil and its moisture content, as well as on the type of crop grown in the soil. Many

fungi can be isolated from the air and from the surfaces of plants and human skin and animal^(1,2,3,4,5,6)fungi responsible for many of the diseases that affect humans, including respiratory-related diseases or skin diseases or nails or hair and fungal toxins produced when the fruits and vegetables infection by fungi and resulting in the damage of human health at the beginning In addition to the economic damage caused by plants and their products in the fields orin stageof stores, as well as damage to preserved foods even in refrigerators and products rich in organic matter, and the most famous types of fungus yeast, which plays an important role in the fermentation process in many of the food ^(7,8,9,10,11,12,13,14,15).

There are many ways to isolate the fungi from their places of preservation and keeping them in pure condition. Different methods of isolation vary and depending on the type of fungus and its environmental needs and the medium in it and several important factors such as the fungi that parasite on the plant externally "can be more easily isolated from the fungi then that grow in or between the tissues of the plant or from air or soil in liquid or solid cultuers ^(9,10,11,12,13,15).

Method and Materials

Two media PDA and SDA were used to growth, isolate and diagnose fungi from regions and sites studied in wasit governorate (air and soil). Using 112 cultivars from PDA media and 53 cultivars from SDA media to purification the fungi that growth and diagnosed it. The study was conducted in the following regions: Kut/ Technical Institute, Kafat, Tamo, Damok, Nu'maniyah, Deboni and Azizia, four sites were selected in each of region and four replicates in each sites.

-1- Isolation fungi from air.2

The PDA medium is prepared and sterilized in the steam sterilizer at 121°C and pressure of 15 psi for a quarter of an hour, it was powered in 9 cm diameter

Number of dishes that fungus appearance in it

Percentage of appearance genus fungus = $\frac{\text{Number of dishes that fungus appearance in it}}{\text{Total number of dishes}} \times 100$

from soil fungi 2-2-Isolation

Collecting the sampling, isolation and diagnosis the fungi-1-2-2

Soil samples from the regions and sites studied were collected from different levels of the surface layer of soil from depth of 10- 30 cm, 1 kg per sample and then brought to the laboratory, mixing different levels of soil depths from which samples were collected separately then making serial dilutions from this samples from 10^1 to 10^5 and select the dilution 10^3 . Took 1 cm³ dilution

petri dishes. The dishes was distributed to seven regions studied and four locations in each region and four replicates in each location then opened it in the air for 15 minutes then closed it and returned to the laboratory and incubated it in the incubator for 72 hours at a temperature of 30 °C. Then the fungal growth were purified on medium SDA and returned to the incubator for 48 hours at the same temperature after that examined the developing fungal growths and was identified on the basis of the external appearance of the growth fungus on media and microscopic characteristics features then classified by using globally approved taxonomic keys^(1,2,3,5,6,7) The percentage emergence of fungi in the regions, sites and replicates was studied are calculated according to the following equation:

10^3 and added to the dishes containing the pre-prepared PDA media using 112 cultivars and 28 cultivars don't add anything to its for control, then were incubated at 30 °C for 72 hours. The growth of fungi in the cultivars were transferred to the SDA media and returned to the incubator again at the same temperature for 48 hours. The developing growth fungal were examined and identified on the basis of morphological and microscopic characteristics and then classified by using globally approved taxonomic keys^(6,5,4).

Finding

Table(1) shows the results obtained after isolating the fungi genus from the air

Serials	Region	Fungi				
	Kut technical 1	Aspergillus 90	Penicillium 75	Rhizopus 10	Ustilago 7	Mucor 9
2	Kafat	94	79	11	5	7
3	Tamos	94	80	11	6	8
4	Damok	92	74	9	5	7
5	Numania	91	76	12	12	8
6	Deboni	89	77	12	10	9
7	Asizia	91	82	13	11	7
Average	91.57		77.57	11.14	8	7.85

* Each number in the table represents the average of four sites in each of the seven regions and four replicates per site.

The results obtained, are shown in table(1) showed that fungus *Aspergillus* was the most common genus of fungi isolated from the air, with a prevalence rate of 91.57% in the region and sites was studied. So this is a danger to human health. causing significant health damage as a result of respiratory infection because this fungus produce large number of conidia spores spread in the air when these spores entry the respiratory system causes the shortness of breath and effects on the lungs by causes aspergillous diseases to it, in addition to the toxins of this fungal, such as Aflatoxine, which produced by this fungus as a secondary metabolite after growing on the food and fruits and vegetables^(16,17,18,19) followed by the genus of the fungus *Penicillium* with an average appearance rate of 77.57%. This fungus is more important too in addition to fungus *Aspergillus* due to the fungal toxins patuline produced by this fungus species expansum when it is found on food especially apple including that fruits and vegetable in refrigerators, because this fungus prefer the moderate temperatures and it can grow in low temperature in the refrigerators,

the fungus *Rhizopus*, *Ustilago* and *Mucor*, followed the *Penicillium* in the percentage of the appearance the rate of appearance 11.14, 8 and 7.85% respectively, and these are all fungi affecting the health of humans, poultry and animals.

The high proportion of the emergence of *Aspergillus* fungus can be explained because it is characterized by the production of a large number of conidia spores that spread in the air in addition to the environment where it is preferred the temperature is high and mild while *Penicillium* prefer moderate or low temperature and it exists even on the food, fruits and vegetables stored in the refrigerators, while the decrease in the proportion of the effect of the emergence of the fungus *Rhizopus* to the lack of production of sporangia and the need for a wet and dark environment of germination and reproduction as well as for *Ustilago* and *Mucor* ^(21,22,23,24)

Table(2) Show the results obtained after isolating the fungi genus from the soil

serials	region	Percentage of appearance genus fungus							
		Fusarium	Cladosporium	Pythium	Phytophthora	Rhizoctonia	Microsporium	Aspergillus	Penicillium
1	Kut technical	38	23	25	19	14	4	9	7
2	Kafat	29	15	11	9	6	15	6	5
3	Tamos	30	13	9	7	6	6	4	4
4	Damok	28	13	9	6	5	7	5	3
5	Numania	39	25	26	22	20	12	10	8
6	Deboni	33	23	26	25	19	16	11	9
7	Asizia	41	23	27	20	20	15	10	9
Avereg		343	19.28	19	15.42	12.85	10.71	7.85	6.42

* Each number in the table represents the average of four sites in each of the seven regions and four replicates per site.

The results were shown in Table (2) refer to that fungus *Fusarium* was the most common of isolates from soil in the surveyed regions and sites at a rate of 33%, followed by the fungus *Cladosporium*, *Pythium*, *Phytophthora*, *Microsporium*, *Rhizoctonia*, *Aspergillus* and *Penicillium*, the reason for the fungus *Fusarium* has a higher appearance rate because *Fusarium* is one of the most abundant soil inhabitant and have three types of

spores produced, while *Aspergillus* and *Penicillium*, is a fungus that spreads in the air^(1,2,3,4,5,8,16,17,,19). The difference in the appearance of fungi in the study regions is due to the fact that the regions from which samples were taken from sites where agricultural soil is endemic by fungi where fungi prefer soil environment and cultivated so the percentage of the appearance of fungi was high in the soil of Azizia and Nu'maniyah and the Institute of

Technical and Deboni.

Conclusions

1-Pollution of the air environment in wassit city with a high percentage of fungi that harmful to human health.

2 - Pollution of the soil environment in wassit city with varying proportions of fungi spores especially the fungi *Aspergillus*, *Microsporum* and *Penicillium* that harmful to human.

3 - The high percentage of the appearance of air polluted fungi in agricultural areas such as Nu'maniyah and Azizia as well as residential areas due to the accumulation of waste, especially fruits and vegetables damaged.

Conflict of Interest :None

Source of Funding :Self

Ethical Clearance: All samples taken after the patients' approval.

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Investigation of DNA Changes in Chronic Myeloid Leukemia (CML) Patients Using Molecular Techniques

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Abstract

Modern molecular techniques were used to identify the damage of DNA molecule. In this study, a high-precision technique was used to identify other genetic changes that lead to cellular changes at the molecular level causing damage and changes to the DNA molecule. We have used complete sequencing technique and information management to compare these markers although we have identified many new forms and differences in genes for chronic myeloid leukemia patients. Those differences that can be identified were easily disaggregated by a simple fractionation of the sequence polymerization reactions (PCR) of NOTCH4 and FAM46A, genes. In addition, the presence of these differences were also assessed in about 30 patients and compared the results with 10 samples of healthy at the end of abstract authors should mention what they are come with conclusion from this work.

Keywords: *Whole exome sequencing, Chronic Myeloid Leukemia, Bioinformatics analysis, PCR reactions.*

Introduction

Chronic myeloid leukemia (CML) is a rare slow increasing blood cancer which begins in the bone marrow, but often moving into the blood sometimes called chronic myelogenous leukemia; chronic granulocytic leukemia, or chronic myelocytic leukemia can be defined as a clonal disease that resulting from an acquired genetic change in a pluripotential hemapoietic stem cell ^[1] In chronic myeloid leukemia (CML), part of the DNA from one a chromosome moves to other chromosomes. This exchange is called the “Philadelphia chromosome”, which results in a reciprocal translocation between the ABL (Abelson Murine Leukemia) gene located on chromosome 9, and the BCR (breakpoint cluster region) gene on chromosome 22 ^[2,3]. This change results in formation of an abnormal gene called *BCR-ABL*, which turns the cell into a CML cell ^[4]. In addition, mutations in the Bcr-Abl kinase domain may contribute to resistance to tyrosine kinase inhibitors (TKIs) in chronic myeloid leukemia patients. CML can be diagnosed by conventional cytogenetic tests that look at chromosomes (pieces of DNA) under a microscope to find any abnormalities. This method takes time, and is not always successful. Therefore, some other molecular approaches have been suggested and used to diagnose and follow

up CML patients’ recovery. However, all these methods have their own limitations and most of them are based on very limited genetic information. Next generation sequencing (NGS) has proven to be an effective tool to identify recurrent, specific mutations in solid tumors and leukemia ^[5]. Whole exome sequencing is one of the NGS approaches that look at the coding regions (Exome) in the genome. Whole exome sequencing (WES) can efficiently identify coding variants and has a wide range of applications in population genetics, genetic disease identification and cancer studies. In whole exome sequencing (WES), DNA samples are fragmented and biotinylated oligonucleotide probes (baits) are used to selectively hybridize to target regions in the genome. After enriching the sample for DNA from the target region, the sample is then sequenced before proceeding to bioinformatics analysis ^[6]. Whole exome sequencing allows the identification of a large number of mutated genes in many types of cancer ^[7]. Therefore, sequencing a wider panel of genes is beneficial in the clinical management of CML patients. As such, the aim of this study is to gain insights into the genetic alterations other than BCR-ABL translocation at the molecular level, which finally causes CML. We will perform whole-exome sequencing of one cases representative of BCR-ABL positive CML in chronic phase of the disease.

Then we will enrich this data with those deposited in the NCBI and ENA databases. After detection of variants, we will assess them in a population of 30 CML patients alongside a population of 10 none cases. At the end, by analyzing our data alongside other published resources and available genomics and transcriptomics data, we will present a system biology level of gene regulation in this disease using bioinformatics approaches

Material and Method

“Data Collection” Patients Selection

Patients have selected according to clinical and laboratory examination. Questionnaires form was filled for each patient. The Questionnaire includes: name, age, gender, date of diagnosis and type of leukemia was achieved. The number of chronic myeloid leukemia was (40) samples. While the range of age in CML was (40-70) years, healthy samples were (10-60) years. Gender of CML 22(male) and 8(female) and healthy 5(male), 5 (female). Five ml of blood has been collected by vein puncture from 40 patients suffering from CML who were admitted to the (Al Reda Hospital) / (The National Center of Hematology) during the periods of May 2017 till September 2018. The disease has been clinically diagnosed by the consultant medical staff at the centre. Each collected blood sample had been dispensed into EDTA tubes for molecular examination. In addition, 10 apparently healthy volunteers used as control had also been included.

Genomic DNA isolation

The genomic DNA isolated from the whole fresh and frozen blood collected in EDTA anticoagulant tubes for molecular studies had been conducted using Wizard (Sambrook *et al.*, 1989). genomic DNA purification kits (Geneaid), following the manufacturer's manual with some modifications. After genomic DNA extraction, agarose gel electrophoresis has been used to confirm the presence and integrity of the extracted DNA.

- Exome sequencing

• Exome sequence analysis

Briefly, raw data were quality controlled, cleaned, aligned and assessed for the presence of insertions, deletions (InDels) and single nucleotide polymorphisms (SNPs) ^[10,11]. For quality control, we have used FastQC^[11] software with default parameters. Trimming

of the adaptors and filtering low quality reads were performed using FASTX toolkits ^[12]. Burrows-Wheeler Aligner (BWA) ^[13]. Software is utilized to map the paired-end clean reads to the reference genome. The original mapping result in BAM format can be obtained. SAMtools is used for sorting the BAM file, and Picard is utilized to mark duplicate reads. Final BAM file can be obtained after these steps. ^[14] GATK software was used to detect variations. We have used ANNOVAR (functional annotation of genetic variants from high-throughput sequencing data ^[15]. To annotate SNPs and InDels, which were includes annotation information from dbSNP, the 1000 Genomes Project and other published databases ^[16]. Annotation contains the variation's position, type, conservation prediction and additional various information.

• Bioinformatics analysis of Exome Seq from different CML patients

The results of exome sequencing were compared across 12 patients obtained in other studies using Integrated Genome Browser (IGB). The information regarding InDels of the CML patients was obtained from European Nucleotide Archive (ENA) and presented. In analyzing, the results using IGB the following criteria were used to find novel CML associated alterations: Experimental techniques that survey an entire genome demand flexible, highly interactive visualization tools that can display new data alongside foundation datasets, such as reference gene annotations. The Integrated Genome Browser (IGB) aims to meet this need. IGB is an open source, desktop graphical display tool implemented in Java that supports real-time zooming and panning through a genome; layout of genomic features and datasets in moveable, adjustable tiers; incremental or genome-scale data loading from remote web servers or local files; and dynamic manipulation of quantitative data via genome graphs. ^[18]

1-PCR verification of the Exome sequence analysis

a. Specific PCR-Primers:

In order to confirm the results obtained from exome sequencing and bioinformatics analysis, PCR reaction was performed using specific primers for the NOTCH4 and FAM46A genes. Table (1).

Table (1). Sequences of primers used in the current study.

Primer	Forward	Reverse	Product size
NOTCH4	5'-TGGGTCTGACCACTGAGACA-3'	5'-ACGTGAGGCTTGCAGCAG-3'	207 bp
FAM46A	5'-CTTGAGCGTGAGTGGTGTGA-3'	5'-GGCGGAGGGTGAAGGGTA-3'	550 bp

b. PCR Reactions and program:

Optimization of PCR reaction was accomplished after several trials. Conclusively, the following programs were adopted. The PCR reaction was carried out as shown in Table (2).

Table (2). PCR Programs for all Tow genes.

Steps	Temperature (C°)	Time	Cycles
Initial denaturation	94	5 minute	1
Denaturation	94	40 second	35
Annealing	62	40second	
Extension	72	45second	
Final extension	72	5 minute	1

b. Analysis of PCR Products:

The PCR products and the ladder marker have been resolved by electrophoresis. 3 µl of loading buffer plus 7 µl of the product were loaded on 2 % agarose gel (2g agarose/100 ml 1X TBE buffer) and run at 100 volts for 40 min. The gel was stained with GEL RED (0.4 µg/ml) solution (0.5 µg/ml). In addition, bands were visualized on UV Tran illuminator and then photographed. DNA ladder (100 bp) has been used to estimate the molecular size of the bands^[17].

Finding and Discussion

Whole-exome sequencing of chronic myeloid leukemia.

The sequencing data including exome reads was provided in fastq format by Novo gene Pairs end reads of 150 nucleotides undergone further analysis (Supplementary materials).

In this study we have compared the presence and absence of such variations in our patient in order to identify of InDels variations with probable association with CML in which obtained with a couple of cases from ENA. All data which were loaded into IGB browser and the positions for these novel InDels were assessed manually”

By analyzing these positions, genes were selected for PCR conformation. These are NOTCH4, and FAM46A. The IGB output for these genes . The full results of IGB for other positions are provided in the supplementary materials. Table (3)

Table (3) The list of novel InDels with no ID in the NCBI human variation databases.

Chromo	POS	ID	ALT	REF	GeneName
4	88536886	.	C	34 nucleotides	DSPP
5	140186981	.	GACA	G	PCDHA4
6	32191658	.	T	TAGCAG CAGCAGC	NOTCH4
8	10467589	.	49 nucleotides	T	RP1L1
6	29910770	.	A	ACCC	HLA-A
6	82461727	.	31 nucleotides	A	FAM46A
6	29910779	.	GCTCC	G	HLA-A

Genomic DNA extraction

Genomic DNA was extracted from blood samples using Wizard Genomic DNA Kit (Promega, KORA) to obtain a pure DNA for PCR amplification. The results of DNA extraction showed that fresh blood samples yielded enough DNA concentrations for PCR amplification.

PCR Analysis:

Using specific PCR primers for each gene. Total DNA shown in figure 1 and table (4). Indicated that a size of pcr product with a molecular weight of about 207 bp for NOTCH4, 500 bp for FAM46A, has been obtained (figure -2).

Table (4) Expected size of PCR products (bp) for each gene in the healthy and CML patients.

Type of gene	Healthy	CML
NOTCH4	200	205
FAM46A	470	500

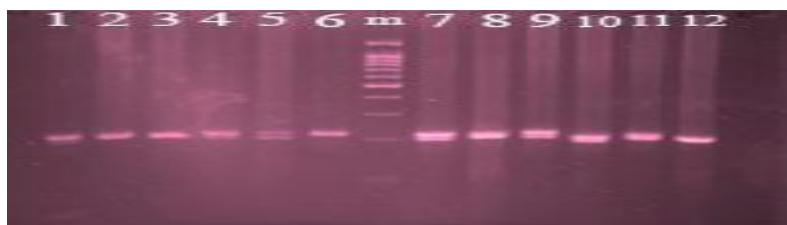


Figure1. PCR products of NOTCH4 gene for CML on a 2 % agarose gel at 100 voltages for (40) min. Lane m 100 bp (marker) Lanes (1,2,3,4,5,6) patient samples and Lanes (7,8,9,10,11,12) control samples for CML size (207) bp

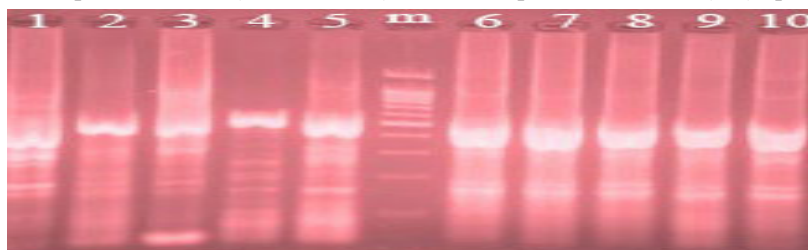


Figure 2. PCR products of FAM64A gene for CML on a 2 % agarose gel at 100 voltages for 40 min. Lane m 100 bp (marker) Lanes (1,2,3,4,5) patient samples and Lanes (6,7,8,9,10,11) control samples for CML size 550 bp.

Conclusions

After analyzing the sequence of data and vital information for CML patients, we selected 2 gene including NOTCH4 and FMA46A to verify PCR. Our results show that this technique is an important technique in the knowledge of candidate genes, as well as in early detection of cancer and further work on the identification of a new marker for early detection of CML. However, further work is needed to demonstrate the relationship between the presence of InDels identified here and the development of CML and its progression.

Source of Finding: Self

Ethical Clearance: Non

Conflict of Interest: Non

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Mobile Games Effects on Visual Acuity of Primary School Students and the Role of Chewable Multivitamins in the Improvement

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Abstract

Children today encounter and utilize technology constantly both at home and in school. Television, DVDs, video games, the internet, cell phones and PDAs – all now play a formative role in many children's development. Excessive use of mobile devices can cause eye problems like myopia (short-sightedness) and amblyopia (lazy eye) in young children. With young children increasingly playing for hours on end with mobile devices such as smartphones and tablets, this might grow to be a problem for them as well. The aim of this study was to assess mobile games effects on visual acuity of primary school students and the role of chewable multivitamins in the improvement. This study assessed 330 students from 4th, 5th and 6th classes of three different primary schools. They were checked regarding visual acuity defects to determine the relationship between these defects and mobile games. The study was conducted at the period [October 2015-April 2017]. Full ocular examination was done, including visual acuity, cycloplegic and non-cycloplegic refraction assessment. Most of the selected sample (69.7%) were normal, while (28.8%) were with impaired visual acuity secondary to refractive errors and (1.5%) without refractive errors. The results showed that there is significant association (strong) between visual acuity and mobile games, 33.5% only of students with normal visual acuity play mobile games, while 77.9% of students with refractive disorders play mobile games.

This strong association is highly related to the time of playing, so that most of defects in visual acuity occurs in students with night time of mobile game playing 52.7%.

Regarding the type of refractive errors, 56.7% was with hypermetropia, 33.78% was myopic and the remaining 9.46% was with astigmatism.

Associated problems with students use of mobile games and refractive errors include wide range of complications, headache occurred in 40.54% of them, 70.3% complaining of reading problems (poor concentration, not clear words ...etc.), red congested eyes in 20.3% and tic with abnormal lid movements in 12.2 % of them. The students with refractive errors subdivided into two groups, the first one received chewable multivitamin while the second group received placebo (nothing) and after 3 months of this clinical trial, there was strong association between refractive errors improvement and chewable multivitamins.

Keywords: Mobile Games ; Visual Acuity ; Primary School ; Chewable Multivitamins ; Improvement.

Introduction

In the past when kids were not in school, was time largely spent roaming the outdoors, playing games with friends and exploring the surrounding area. ⁽¹⁾

Kids and mobiles are nearly inseparable these days. With many school-age kids and even preschoolers

spending hours in front of a mobile every day, it's worth considering what effects mobile games might have on your children's eyes and their vision ⁽¹⁾. Most mobile games require lengthy involvement to finish any particular game, forcing the player to stay focused on the screen during their playtime ⁽²⁾. In 2009, 29 percent of American children ages 8 to 18 had their

own laptop computer, and kids in grades 7 through 12 reported spending an average of more than 90 minutes a day sending or receiving texts on their cell phones ⁽²⁾. Many pediatric eye doctors believe that heavy computer and mobile use among children puts them at risk for early myopia. Recent research appears to confirm that fear ⁽³⁾. A large study conducted by the National Eye Institute and published in the December 2009 issue of *Archives of Ophthalmology* found that the prevalence of nearsightedness among Americans has increased from 25 percent to 41.6 percent of the population over the past 30 years — an increase of more than 66 percent⁽²⁾

Sitting for hours in front of a mobile screen stresses a child's eyes because the mobile forces the child's vision system to focus and strain a lot more than any other task. This can put children at an even greater risk than adults for developing symptoms of mobile vision - syndrome⁽⁴⁾. Mobile eye effects include visual acuity impairment (refractive errors), eye strain, crossed eye (squint), headache, defective eye nourishment (dry eye), High Energy Visible light effects (HEV) and blindness ⁽⁴⁾. Today it is a "near-point world," and parents need to be aware of the vision problems associated with mobile work. Mobile use demands fine motor skills from young eyes that are not well developed. Only when the visual system matures is a child better able to handle the stress of a mobile on that system. ⁽⁵⁾

Blue light is the highest energy wavelength of visible light. The "energy" is able to penetrate all the way to the back of the eye, through the eyes' natural filters. A good way to think of how eye supplements work is that they act as natural sunglasses and shield eyes from harmful rays – like UV rays from the sun or blue light rays from digital devices, so it's like applying sunscreen to the skin to protect it from sun damage. Like sunglasses and sunscreen, eye supplements are best used regularly for effective protection. ⁽⁵⁾

Patients and methods

The current study is across sectional- clinical trial study to assess mobile games effects on visual acuity of primary school students and the role of chewable multivitamins in the improvement. The study included 330 students from 4th, 5th and 6th classes of three different primary schools in Tikrit city. They were checked regarding visual acuity defects to determine the relationship between these defects and mobile games. The study was conducted at the period [October 2015-April 2017]. The clinical trial part of study was done by giving one group of the students with refractive errors chewable multivitamins "Happy Gummy[®]" is a formulation carefully studied to provide a fusion of essential Vitamins, Minerals, Calcium, Iron, Zinc, Folic acid and natural fruit juice flavors in a delicious gummy», one piece per day for two months, while the other group has been given placebo.

Data collected included general information in addition to full ocular examination was done, including visual acuity, cycloplegic and non-cycloplegic refraction assessment. Then data summarized and presented by simple tables and figures, and analyzed by using ANOVA test.

Finding

Three hundred thirty (330) students from 4th, 5th and 6th classes of three different primary schools in Tikrit city were chosen randomly and checked for the presence of refractive errors in relation to their use of mobile games.

(69.7%) of students were normal in relation to visual acuity, (28.8%) with defective visual acuity due to refractive errors and only (1.5%) impaired vision due to non-refractive errors (trauma, cataract, retinal problems, glaucoma ...etc.).

The results showed no significant relation between school class and visual acuity status. (p-value=0.207).

Table -1- Sample distribution according to school class.

Visual acuity	School Class							Total
	4th		5th		6th			
	Number	%	Number	%	Number	%	Number	%
Normal	89	38.7	79	34.3	62	27	230	69.7
Refractive Errors	30	31.6	40	42.1	25	26.3	95	28.8
Non refractive errors	0	0	2	40	3	60	5	1.5
Total	119	36.0	121	36.7	90	27.3	330	100

Table -2- The relation between visual acuity and gender.

Visual acuity	Gender						Total
			Male		Female		
	Number	%	Number	%	Number	%	
Normal	96	41.7	134	58.3	230		69.7
Refractive Errors	55	57.9	40	42.1	95		28.8
Non refractive errors	3	60	2	40	5		1.5
Total	154	46.7	176	53.3	330		100

Regarding gender association, significant association was present between male gender and the presence of refractive errors. (**P-value=0.025**).

Table -3- The relation between mobile games and visual acuity.

The results showed that there is significant association (strong) between visual acuity and mobile games, **33.5%** only of students with normal visual acuity play mobile games, while **77.9%** of students with refractive disorders play mobile games.

Visual acuity	Mobile Games						Total
			Present		Absent		
	Number	%	Number	%	Number	%	
Normal	77	33.5	153	66.5	230		69.7
Refractive Errors	74	77.9	21	22.1	95		28.8
Non refractive errors	4	80	1	20	5		1.5
Total	155	47	175	53	330		100

Table -4- The relation between visual acuity and hours of playing mobile games.

Visual acuity	Hours of playing								Total	
	< 1 hour		1-< 2 hour		2-< 3 hour		3-< 4 hour			
	Number	%	Number	%	Number	%	Number	%	Number	%
Normal	54	70.1	14	18.2	6	7.8	3	3.9	77	49.7
Refractive Errors	5	6.8	10	13.5	23	31.1	36	48.6	74	47.7
Non refractive errors	2	50	1	25	1	25	0	0	4	2.6
Total	61	39.4	25	16.1	30	19.4	39	25.1	155	100

According to the results, only 3.9% of students with normal visual acuity play mobile games for more than 3-4 hours, while this percentage was 48.6% of students with refractive problems, so that there is strong association between visual acuity status and hours of mobile games playing (**p-value= 0.05**).

This strong association is highly related to the time of playing, so that most of defects in visual acuity occurs in students with night time of mobile game playing **52.7%**. (**p-value=0.05**).

Table -5- Relation between visual acuity and time of playing mobile games.

Visual acuity	Time of playing						Total	
	Day		Night		Day and night			
	Number	%	Number	%	Number	%	Number	%
Normal	48	62.3	17	22.1	12	15.6	77	49.7
Refractive Errors	5	6.8	39	52.7	30	40.5	74	47.7
Non refractive errors	3	75	1	25	0	0	4	2.6
Total	56	36.1	57	36.8	42	27.1	155	100

Regarding the type of refractive errors, **56.7%** was with hypermetropia, **33.78%** was myopic and the remaining **9.46%** was with astigmatism.

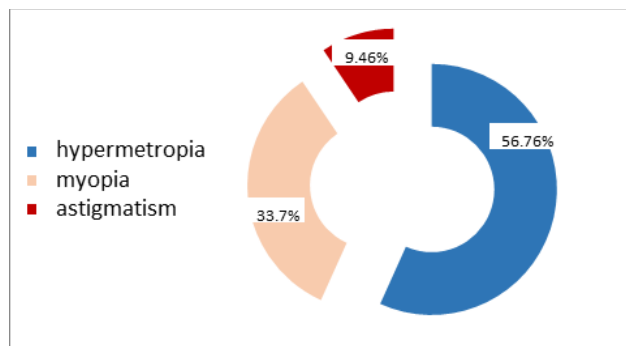


Figure -1- Refractive error distribution according the type.

Associated problems with students use of mobile games and refractive errors include wide range of complications, headache occurred in 40.54% of them, 70.3% complaining of reading problems (poor concentration, not clear words ...etc.), red congested eyes in 20.3% and tic with abnormal lid movements in 12.2 % of them.

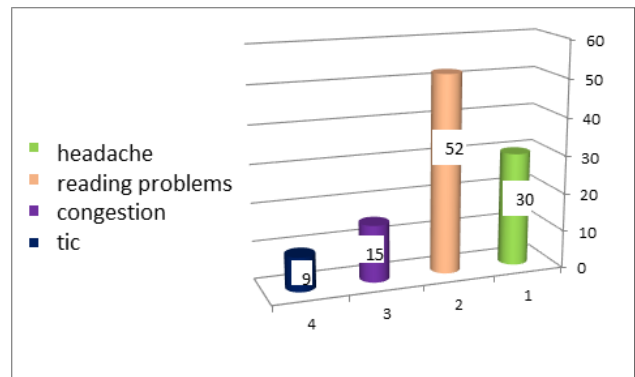


Figure -2- Associated problems to refractive errors.

The students with refractive errors subdivided into two groups, the first one received chewable multivitamin while the second group received placebo (nothing) and after 3 months of this clinical trial, there was strong association between refractive errors improvement and chewable multivitamins. (**p-value=0.05**).

Table -6- Refractive errors & associated problems improvement with chewable multivitamins after 3 months follow-up.

Improvement	Multivitamins		Placebo		Total	
Excellent	18	81.8	4	18.2	22	29.7
Good	12	66.7	6	33.3	18	24.4
Fair	5	20	25	80	30	40.5
Weak	2	50	2	50	4	5.4
Total	37	50	37	50	74	100

Discussion

An estimated 3.35 billion personal computers, tablets and mobile phones were shipped around the world in 2016. With new products hitting shelves every day, the market for digital devices continues to grow. this means more time spent looking at electronic screens and digital content.⁽⁶⁾

The world's reliance on electronics has proven to be beneficial in many ways but poses a problem for the eyes. nearly 70 percent of U.S. adults experience digital eye strain as a result of the growing use of these devices.⁽⁶⁾

New research also suggests that overexposure to blue light, also referred to as high-energy visible or HEV light, may contribute to vision problems such as cataracts and age-related macular degeneration (AMD). long-term implications are just now being studied, but the short-term impact of digital eye strain affects individuals on a daily basis.⁽⁶⁾

In this cross sectional and clinical trial study, the demonstration of mobile games effects on visual acuity of primary school students and the role of chewable multivitamins in the improvement. The results showed that (69.7%) of students were normal in relation to visual acuity, (28.8%) with defective visual acuity due to refractive errors and only (1.5%) impaired vision due to non-refractive errors and these results similar to that of Cespedes.⁽⁷⁾

The results showed that there is significant association (strong) between visual acuity and mobile games, 33.5% only of students with normal visual acuity play mobile games, while 77.9% of students with

refractive disorders play mobile games and these results slightly different from that of Gartner.⁽⁸⁾

Only 3.9% of students with normal visual acuity play mobile games for more than 3-4 hours, while this percentage was 48.6% of students with refractive problems, so that there is there is strong association between visual acuity status and hours of mobile games playing. This strong association is highly related to the time of playing, so that most of defects in visual acuity occurs in students with night time of mobile game playing 52.7%. The same fact was mentioned by Cathy.⁽⁹⁾ Regarding the type of refractive errors, 56.7% was with hypermetropia, 33.78% was myopic and the remaining 9.46% was with astigmatism.

Associated problems with students use of mobile games and refractive errors include wide range of complications, headache occurred in 40.54% of them, 70.3% complaining of reading problems (poor concentration, not clear words ...etc.), red congested eyes in 20.3% and tic with abnormal lid movements in 12.2 % of them and this similar to other studies.⁽¹⁰⁾

The students with refractive errors subdivided into two groups, the first one received chewable multivitamin while the second group received placebo (nothing) and after 3 months of this clinical trial, there was strong association between refractive errors improvement and chewable multivitamins.

Conclusions

- Significant relationship between mobile games and refractive errors in primary school age students.
- This relationship is more in males more than females.

- There is strong association between the duration of playing and mobile eye related problems.

- Using of chewable multivitamin is with significant beneficial effects.

Source of Finding: Self

Ethical Clearance: Non

Conflict of Interest: Non

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Torque Teno Virus (TTV) as a Risk Factor in Hemodialysis Process in Kirkuk.

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Abstract

Patients undergoing hemodialysis are at increased risk of infection with blood-borne viruses, especially hepatitis C virus (HCV), hepatitis B virus (HBV) and Torque teno virus (TTV). The present study investigated these issues and the possibility of nosocomial transmission among patients undergoing hemodialysis. A cross sectional study was carried out on 80 patients with end stage renal disease (ESRD) undergoing hemodialysis (HD) and 20 blood donors as control who admitted to blood bank for blood donation were enrolled in this study in Kirkuk province from 15th of August 2018 to 15th of February 2019. **Result:** The prevalence of TTV DNA was detected in those TTV Ag seropositive and some seronegative dialysis patients, genetic analysis was done using the primers from N22 region of TTV. The study showed that 15% of HD patients had TTV DNA. The study showed that the highest rate of TTV infection (30%) was found in patients under dialysis for more than 3 years. On the other hand, the study displayed that the highest rates of TTV infections were recorded in HD patients who previously received blood for more than 6 times (58.33%). The current study revealed that there was no significant association between TTV infection and level of ALT and AST in HD patients.

Key words: HD, TTV, CKD, ESRD, HCC.

Introduction

The use of hemodialysis as renal replacement option in end-stage renal disease (ESRD) has provided better life expectancy in those patients, but maintenance hemodialysis is a high-risk environment for blood-borne viruses especially HCV and HBV infections⁽¹⁾. Patients with chronic renal failure are at risk of acquiring microorganisms and developing infections. The most common etiologic agents are bacteria and viruses, such as the Torque teno virus, TTV was discovered as a possible cause of posttransfusion hepatitis in humans in 1997 in Japan⁽²⁾. Currently, the Anelloviridae family comprises about 68 species, which are non-enveloped, circular, single-stranded DNA (ssDNA) viruses with a genome of 2.1–3.9 kb in length classified into 12 genera⁽³⁾. Torque teno virus is a human-infected virus that is present ubiquitously in nature. Globally, it infects up to 95% of the healthy individuals without any clinical manifestations⁽⁴⁾. Spelled-out names for TTV was approved as ‘Torque teno virus’ by the International Committee on Taxonomy of Viruses

(ICTV). These acronyms were devised to incorporate ‘torques’ (necklace) and ‘tenuis’/‘teno’ (thin) into the established TTV name. Some researchers still refer to TTV as ‘transfusion transmitted virus’, based on the initials of the original patient⁽⁵⁾. Torque teno virus has a 3.8kb circular genome of ssDNA. One third of the genome is a non-translated region (NTR) and has a high degree of similarity within extremely divergent TTVs⁽⁶⁾. Human and porcine anelloviruses share the same genomic structure, which consists of at least four presumed open reading frames (ORFs), as well as a short stretch of high GC content in the untranslated region⁽⁶⁾. The ORF1 protein probably serves as replicase and represents the major structural protein of TTV, because its N-terminus region contains a highly basic stretch consistent with other circoviral capsid proteins. Phosphorylation of the ORF 2–4 product on frame 2, derived from the 1.2kb spliced mRNA using an expression plasmid, has a similarity to NS5A of HCV. A transgenic mouse expressing a TTV product mainly in kidneys and suffering from a nephrotic syndrome has been reported⁽⁷⁾. Wherever it replicates, TTV is shed

into the bloodstream in relatively large quantities. Following transfusion of uninfected patients with contaminated blood, TTV became detectable in plasma in one to few weeks⁽⁸⁾. The concentration of TTV is 10–100 times greater in bile than in peripheral blood, suggesting replication of TTV in the liver⁽⁵⁾. The genome of TTV replicated by a rolling-circle mechanism⁽⁹⁾. TTV prevalence and clinical significance are being assessed worldwide, however its relationship with progression to severe liver disease and hepatocellular carcinoma (HCC) remain controversial, although the serological prevalence of TTV infection in healthy blood donors was lower than that in patients with fulminant or chronic cryptogenic liver diseases⁽¹⁰⁾.

Materials and Method

A cross sectional study included 80 patients with end stage renal disease (ESRD) undergoing hemodialysis (HD) and 20 blood donors as a control group who were apparently healthy who admitted to blood bank in Kirkuk province in northern Iraq from 15th of August 2018 to 15th of February 2019. Blood samples were withdrawn with a vacutainer set to exclude air contamination. Samples were stored at – 20°C until processing.

Torque Teno Virus(Ag) serology

A sandwich enzyme-linked immunosorbent assay (ELISA) was used to qualitatively analyze Torque tenovirus (TTV) in human serum, plasma or other biological fluids (My BioSource (USA), Cat. No: MBS9313728).

Isolation of TTV DNA

For detection of TTV DNA in HD patients and the control group, 80 HD patients were included (31 samples of TTV Ag positive by ELISA plus 49 sample from TTV Ag negative by ELISA) and 20 samples from the control group were prepared and stored for later nested PCR, viral DNA was extracted from 200 µl serum stored at 20°C using QiAmp Mini Elute viral spin kit (Qiagen, Germany) following manufacturer's instruction. The nucleic acid was eluted in 30 µl of elution buffer supplied with the kit. TTV-DNA was detected by nested PCR using N22 specific primers. First round amplification was performed with forward primer NG059-5'-ACAGACAGAGGAGAAGGCAACATG-3' and reverse primer NG063-5'-CTGGCATTTCACATTTCCTCAAAGTT-3' for

5 min at 95°C (initial denaturation) followed by 50 cycles of denaturation at 95°C for 1 min, annealing at 62°C for 1 min, and extension at 72°C for 1 min with final extension at 72°C for 7 min. The second round of PCR was performed using forward primer NG061-5'-GGCAACATGYTRTGGATAGACTGG-3' and reverse primer NG063-5'-CTGGCATTTCACATTTCCTCAAAGTT-3' following same conditions as used for first round amplification in a thermocycler. Five µl of the PCR product was electrophoresed on 2% agarose gel and stained by ethidium bromide safe nucleic acid staining solution for the detection of N22 region of TTV.

Biochemical tests

Liver function tests including aspartate aminotransferase (AST) & alanine aminotransferase (ALT) were performed on autoanalyzer Roche-Reflotron.

Statistical Analysis

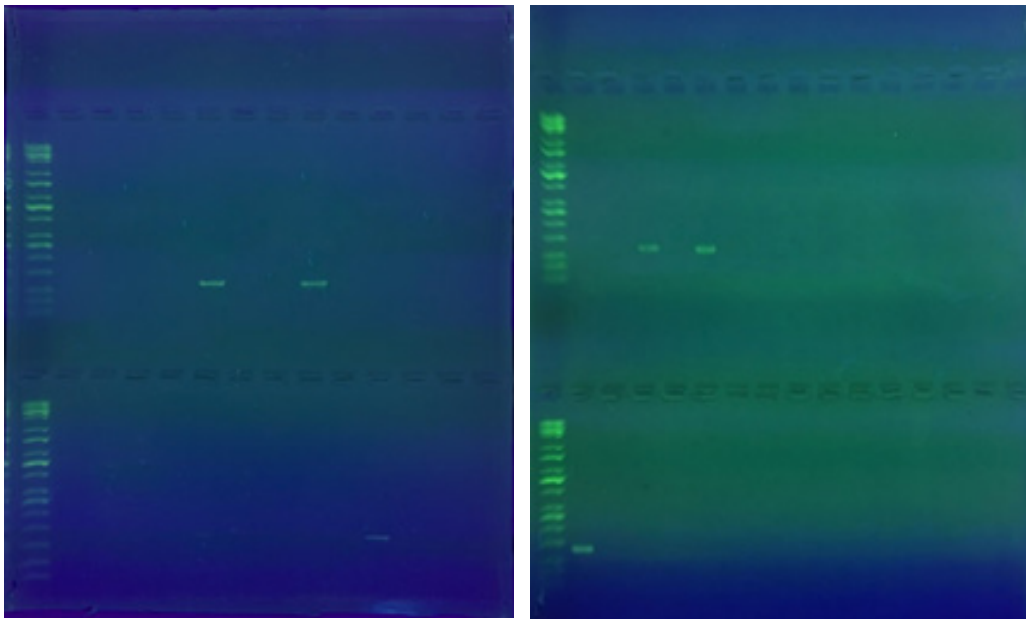
Computerized statistical analysis was performed using SPSS (Statistical Package for Science Services). Comparison carried out using Chi-square (χ^2) and probability (P value).

Finding

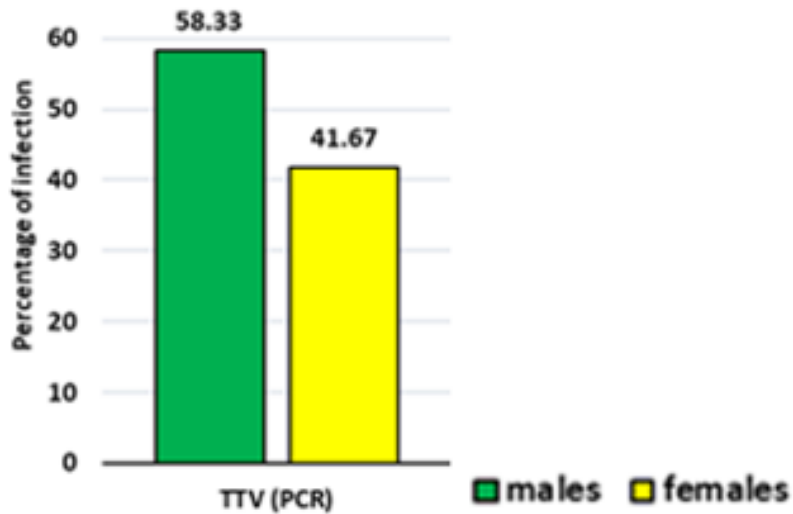
This study was concluded to reveal the rate of TTV infection in a population of CRF patients on maintenance HD, in Kirkuk general Hospital. Hemodialysis is carried out in those patients who have developed CKD-stage IV and ESRD. In order to find out the prevalence of TTV infection, the sera samples from 80 CRF patients were assayed for the presence of TTV-DNA using polymerase chain reaction (PCR) and amplifying N22 region of TTV-genome. For detection of TTV DNA, 80 HD patients were included (31 samples of TTV Ag positive by ELISA plus 49 sample from TTV Ag negative by ELISA) and 20 samples from the control group were prepared and stored for later nested PCR. The present study revealed that TTV DNA was detected in 15% of hemodialysis patients, no one of control group had TTV DNA, as shown in Table 1 and Figure 1.

Table 1: Detection of TTV DNA in HD patients and the control group by PCR.

Age groups (years)	Total No. (80)	TTV positive (PCR) (n:12)		<i>P. value</i> 0.06
		No.	%	
15-24	12	4	33.33	Mean age (years): 34.33
25-34	10	3	30	
35-44	12	3	25	
45-54	20	1	5	
55-64	14	1	7.14	
65-75	12	0	0	

**Figure 1: Polymerase chain reaction product the band size 271 bp. The product was electrophoresis on 2% agarose at 5 volt/cm². 1x TBE buffer for 1:30 hours. N: DNA ladder (100).**

In the present study, TTV infection was found in a higher rate in males (58.33%) than that in females (41.67%)..
Figure 2.

**Figure 2: Distribution of TTV infection according to gender.**

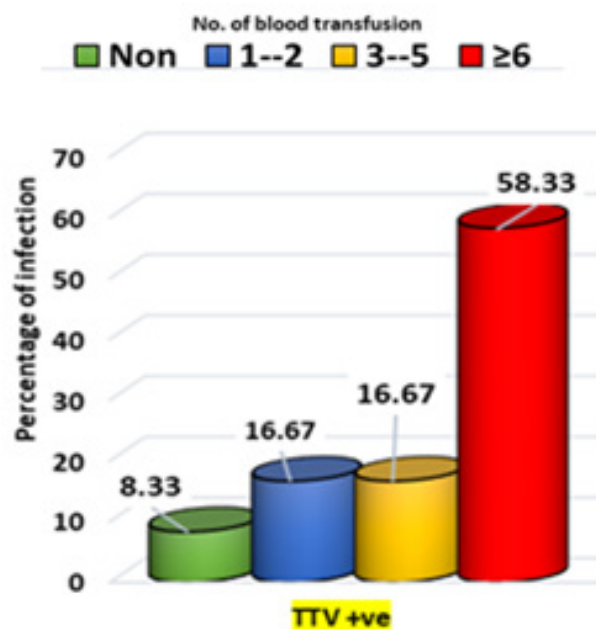
The study revealed that the highest rate of TTV infection (33.33%) was found in HD patients within the age group 15-24 years.. Table 2.

Table 2: Relation of TTV infection with age of patients under hemodialysis.

Age groups (years)	Total No. (80)	TTV positive (PCR) (n:12)		<i>P. value</i> 0.06
		No.	%	
15-24	12	4	33.33	Mean age (years): 34.33
25-34	10	3	30	
35-44	12	3	25	
45-54	20	1	5	
55-64	14	1	7.14	
65-75	12	0	0	

The study displayed that there was a significant relation of TTV infections with number of blood transfusion in HD patients and the highest rates of TTV infections were recorded in HD patients who previously received blood for more than 6 time (58.33%)and the lowest rates were found in patients who didn't receive blood previously..Figure 3.

Figure 3: Relation of TTV infections with number of blood transfusion in HD patients.



The study showed that the highest rate of TTV infection (30%) was found in patients with dialysis for more than 3 years and rate of TTV infection was increased with increasing of dialysis duration. The result was highly significant,as seen in Table 3.

Table 3: Relation of TTV infection with duration of hemodialysis.

Duration of hemodialysis (year)	Total No. (80)	TTV (PCR)				<i>P. value</i>
		Positive (n:12)		Negative (n:68)		
		No.	%	No.	%	
≤ 1	26	0	0	26	100	0.007
2-3	54	6	11.11	48	88.89	
>3	20	6	30	14	70	

The current study revealed that there was no significant relation between TTV infection and mean level of ALT and AST in HD patients..Table 4.

Table 4: Level of ALT and AST in HD patients and the control group

Parameters		HD patients		Control group (n:20)	P. value	
		TTV +ve (n:12)	TTV -ve (n:68)		(ANOVA)	TTV +ve vs. TTV -ve
ALT* (IU/ml)	Mean ± SD.	18.13 ± 7.91	13.57 ± 10.71	10.17 ± 2.89	0.22	0.32
AST** (IU/ml)	Mean ± SD.	14.07 ± 5.67	13.2 ± 9.62	10.41 ± 2.42	0.48	0.53

Discussions

Infections are the major causes of mortality and morbidity in HD patients. Hence, the patients with chronic renal failure undergoing HD are at risk of TTV infection and suffering from mentioned complications⁽¹¹⁾. It was assumed that TTV may represent as one possible component of viruses causing non-A-G hepatitis. This assumption was also reinforced by the findings that TTV is predominantly present in liver tissue in higher levels as compared to its level in blood or other tissue⁽¹²⁾. The present study demonstrated that the Genetic analysis of TTV by nested PCR using the primers from N22 region of TTV, showed that 15% of HD patients had TTV DNA (+ve PCR). The study demonstrated that 38.71% of HD patients who were positive by ELISA were positive by PCR and no one HD patients with negative ELISA and the blood donors were positive by PCR. The present results were in agreement with several studies done earlier. Takemoto *et al*⁽¹³⁾ reported that the frequency of TTV infection can vary with the genomic region tested, as well as with the geographic location studied, and group of dialysis patients showed a TTV positivity rate of 17%. Nishizawa *et al*⁽¹⁴⁾ reported that the frequency of TTV infection increased to 36% in HD patients. The frequency of TTV infection in patients on maintenance dialysis varies widely; Ibrahim *et al*⁽¹⁵⁾ reported that the prevalence of TTV infection in HD Egyptian patients was 45% compared with 7% in healthy control.

The present study revealed that the highest rate of TTV infection (33.33%) was found in HD patients

within the age group 15-24 year. Abu-Odeh *et al*⁽¹⁶⁾ also showed that an association exists between TTV viremia and age ($P < 0.05$). In the present study, males had a higher rate of infection with TTV (58.33%) comparing with females. Spandoleet *et al*⁽¹⁷⁾ reported that there was no difference in the distribution of TTV in males and females. On the other way, Rinonceet *et al*⁽¹⁸⁾ found that DNA positivity was not associated with age, gender or any of the risk factor analyzed in study.

The study showed that the highest rate of TTV infection (30%) was found in patients under dialysis for more than 3 years and rate of TTV infection was increased with increasing of dialysis duration, the result was highly significant. Akbari *et al*⁽¹⁹⁾ reported no significant association between TTV infection and a wide variety of dialysis factors including times of hemodialysis. Irshad *et al*⁽²⁰⁾ showed that the median time on HD prior to entry to this study was 7 months, the same duration for TTV-positive patients was 7 months. Hemodialysis patients are at high risk for viral hepatitis infections due to the high number of blood transfusions and prolonged vascular access⁽²¹⁾.

The present study displayed that there was a significant relation of TTV infections with number of blood transfusion in HD patients and the highest rates of TTV infections were recorded in HD patients who previously received blood for more than 6 time (58.33%) and the lowest rates were found in patients who didn't receive blood previously. A study reported that history of blood transfusions as well as the number of blood

transfusions were strongly associated with viral hepatitis infection at baseline, but not with new infections. Prior to the introduction of effective screening of blood donors, blood transfusions were recognized as the leading source of HCV infection and some of these infections may have been acquired before adequate screening was introduced^(22,23).

The current study revealed that the highest mean level of ALT and AST were recorded in HD patients with TTV infection 18.13 IU/ml and 14.07 IU/ml respectively comparing with patients without TTV infection and the control group. Although the result was non-significant. That agree with Irshad *et al*⁽²⁰⁾ who reported that analysis of renal function parameters in HD patients with TTV infection didn't show a significant change in renal dysfunction. This showed that TTV infection as such does not damage renal tissue or cause renal dysfunction. Yet, the investigation of TTV prevalence in healthy individuals is required to identify whether transfusion or dialysis are associated with higher TTV prevalence. Besides, even though TTV infection did not change AST and ALT enzymes in thalassemic patients, further studies analyzing the potential role of TTV in post-transfusion hepatitis are recommended⁽¹²⁾. Importantly, Akbari *et al*⁽¹⁹⁾ reported no significant association was found between TTV infection and liver enzymes (*P* value 0.018). Such liver enzyme abnormalities were reported in TTV-infected patients on hemodialysis emphasizing the possibility of liver dysfunction caused by TTV infection⁽²⁴⁾.

Conclusion

The study concluded that infection with TTV was found in HD patients, and revealed that correlated with multiple blood transfusion and duration of HD. The study showed that normal levels of liver enzymes were common in HD patients infected with TTV alone, in contrast to patients with hepatotropic viruses.

Conflict of Interest : Nil

Source of Funding : Self

Ethical Clearance : Taken from patients

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The Relative Contribution of Residual Renal Function to Phosphate and Calcium Metabolism in Maintenance Hemodialysis Patients

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Abstract

Background: The preservation of residual renal function was associated with improved calcium and phosphate metabolism and improvement of mortality and morbidity in hemodialysis patients. The aim of the study: For assessment the achievement of the Kidney disease outcome quality initiative guidelines for appropriate serum calcium, serum phosphate, calcium by phosphate product by product and serum parathyroid hormone and the contribution role of residual renal function for that achievement. Patients and Methods: cross section study of (50) hemodialysis patient patient who divided in to two group, anuric patients and those with preserved then head to head comparison between them for the achievement of kidney disease outcome quality initiative guidelines, were P. value above (0.05) was statistically not significant. Finding : There was a difference although statistically not significant it appeared that patients, anuric group mean Serum phosphate = (6.8 ± 1.2) mg/dl vs preserved residual renal function group = (6.01 ± 1.85) . kidney disease outcome quality initiative guidelines achievement was 25% versus 43.3% respectively. P. value= 0.75. Anuric group mean Serum calcium = (7.4 ± 1.2) mg/dl versus (7.6 ± 1.39) and kidney disease outcome quality initiative guidelines achievement for anuric patients = 30% versus 30%. P. value= 0.18. So anuric had higher serum phosphate and lower serum calcium levels. Conclusion: The preservation of residual renal function was beneficial for hemodialysis patients.

Key words: (RRF): Residual Renal Function. HD: Hemodialysis, SPKT/V(Single pool KT/V), PTH (Parathyroid Hormone), ca (calcium), po4 (phosphate), CKD (chronic kidney disease) and GFR (glomerular filtration rate).

Introduction

Chronic kidney disease (CKD) is defined as kidney damage or (GFR < 60 ml/min. /173m² For three or more months. CKD regardless of its etiology considered as progressive condition) (1), Measures that used for preservation of (RRF) should be continued even after initiation of (HD)(2)

(RRF) even < 5ml/ min considered important for fluid and solute balance, the residual urea clearance of 2-3 ml/ min is equivalent to spkt/v of 0.5 - 0.75(3). (RRF) improve phosphate, calcium $ca \times po_4$ by product and S. PTH (4). Improved survival (5), nutritional status (6), higher hemoglobin level (7), quality of life (8) and status with left ventricular hypertrophy (9). Most patients

started on (HD) still had (RRF) but by the end of the first year the majority of them would loss (RRF). Factors associated with loss of RRF include: Hypotensive and hypertensive episodes during (HD), volume depleting states, proteinuria dialysis modality loss of (RRF) occur in (HD) more than peritoneal dialysis, radio contrast media (10), nephrotoxic agents (11), the use of bio-in compatible membrane (12).

Metabolic abnormalities for calcium and phosphate metabolism can increase mortality and morbidity, cardiovascular complications in addition to metabolic bone disease (12, 13, 14).

For these reasons kidney disease outcome quality initiative (K/DOQI) guidelines for appropriate levels

suggested S. phosphate (3.5 - 5.5) mg/dl, calcium (8.4 - 9.5) mg dl, $Ca \times P$ product < 55 mg²/ dl² and S. PTH (150 - 300) pg/ ml (15). Worldwide only minority of the patients appeared to had Laboratory data that falls within these guidelines(10).

The aim of the study for assessment of the percentages of the patients on (HD) who achieved the values that fall within the K/DOQI guidelines for P_{04} , Ca , $Ca \times P$ by product and S. PTH and to assess the role of (RRF) for improvement of minerals metabolism by comparison of two groups of the patients with and without (RRF).

Patients and Method

This cross section study was done in the dialysis unit, Baghdad teaching hospital. (60) patient were randomly taken, (50) of then enrolled in the study while (9) patient excluded because of incomplete laboratory data and (1) because of history of renal allograft failure. All patient asked about age, weight (pre and post dialysis), duration of their dialysis treatment (in months) and underlying cause of end stage renal disease (ESRD). Patients divided in to two groups, anuric patients (urine output < 100 cc/24 hour (insignificant (RRF) and those with preserved (RRF) according to the definitions of K/DOQI (17). Glomerular filtration rate (GFR) was estimated by modification of diet in renal disease (MDRD) equation (18) and hemodialysis adequacy by Daugirdas formula (20) in addition to use of corrected calcium equation (19).

The two group was compared according to the K/DOQI guidelines achievement for S. P_{04} , S. Ca, $Ca \times P$ by product and S. PTH and compare HD adequacy for the two group, were P. value above (0.05) was statistically not significant.

Statistical Analysis: By using SPSS Y16, 3, 1, US. Software for windows, data of all patients in both groups (Preserved RRF and loss of RRF) we wrote and analyzed with appropriate statistical test, student t- test was used to compare quantitative data, chi-square and Fisher exact tests were used, Spearman rank correlations and corresponding P- values were used to evaluate the relationships between ordinal and other variables and to examine the significance of difference between the study groups regarding the achieve of K/DOQI guidelines. All data were normally distributed and showed a Gaussian distribution ($p = 0.05$ on test of normality. In all analysis

the level significance set at 0.05 statistically was no significant difference between groups.

Finding

The total number of patient in the study = 50, (31) female, (19) male, anuric patients group= 20, (15) female, 5 (male), mean age = (38.05 ± 1.9) year, mean weight = (59.3 ± 11.9) kg, mean duration of dialysis in months = (24.33 ± 24.2) , spkt/v mean = (0.91 ± 0.2) , mean S. Ca = (7.4 ± 1.2) mg/ dl, mean S. phosphate = (6.8 ± 2) mg/ dL, mean S.PTH = (292 ± 231) pg/ ml, mean Ca by P_{04} = (51.1 ± 19.3) mg²/ dL².

Patient with preserved (RRF) No = 30 patient, (14) male, (16) female, mean age = (53 ± 13.39) year, mean weight = (65.3 ± 13.3) kg, mean duration of dialysis = (14.6 ± 15.26) month, spkt/v = (0.78 ± 0.20) , GFR = (9.8 ± 4.8) ml/min S. phosphate = (6.01 ± 1.85) , mean S. Ca = (7.6 ± 1.39) , mean S. PTH = (239 ± 209) and mean $Ca \times P_{04}$ = (45 ± 13.02) . causes of ESRD was shown on table No (4). The distribution of patients groups for achievement of K/DOQI guidelines as the following.

The anuric patient group No = 20, (6) patients achieved K/DOQI target for s. Ca , the patients with preserved RRF No = 30, (9) patients of them achieved K/DOQI target for S. Ca = 30% vs 30% for anuric patients. There's no significant difference P. value = 0.75.

There's no significant difference between the two groups for achievement of S. phosphate target (25%) of 20 anuric patient versus (43.3%) of 30 patient, preserved (RRF), P. value = 0.18.

There's no significant difference between the two groups for achievement of $Ca \times P_{04}$ product target, 60% of anuric patients versus 76.7% of patient with preserved (RRF) P. value = 0.304.

There's no significant difference for achievement of the target for S. PTH, zero percent of anuric patients versus (16.7%) of patient with preserved RRF, P. value = 0.14. There was significant difference in hemodialysis adequacy mean spkt/v for loss of RRF = 0.91 ± 0.25 VS. 0.78 ± 0.20 .

P. value = 0.0472, while dialysis adequacy for all (50) patient = 0.83 ± 0.22 , All of the patients characteristics was tabulated.

Table (1) Demographic and biochemical characteristic of patients with loss RRF (GFR was negligible), N = 20

Descriptive Statistics	Range	Mean \pm S. D.
Age years	15-60	38.05 \pm 11.9
Weight kg	38-107	59.3 \pm 15.0
Duration on dialysis months	1-72	24.33 \pm 24.2
SPKT/v	0.47-1.3	0.91 \pm 0.2
Corrected Ca	5-9.35	7.4 \pm 1.2
P04	3.4-9.8	6.8 \pm 2.0
Ca by P04 product	20.5-90.4	51.1 \pm 19.3
PTH	7.1-511	292 \pm 231

Table (2) Demographic and biochemical characteristics of patients with preserved RRF, N= 30

Descriptive Statistics	Range	Mean \pm S.
Age years	22-71	53.8 \pm 13.39
Weight kg	41-94	65.3 \pm 13.3
Duration on dialysis months	1-48	14.6 \pm 15.26
SPKT/V	0.33-1.18	0.78 \pm 0.20
eGFR	4.53-22.62	9.8 \pm 4.8
P04	3.3-9.7	6.1 \pm 1.85
Corrected Ca	5.32-11.112	7.6 \pm 1.39
Ca by P04 product	20.7-69.7	45.8 \pm 13.02
PTH	2.9-550	239.9 \pm 209

Table (3) Distribution of Patients by gender

Patients Groups	No	Gender	Frequency	Percent
Loss RRF	20	Male	5	25 %
			15	75 %
			20	100 %
Preserved RRF	30	Male	14	43.3 %
			16	56.7 %
			30	100 %

Table (4) Causes of Renal failure among patients groups

Cause	Loss of RRF	Preserved RRF	Total	Percent
Unknown	6	8	14	28 %
DM	1	10	11	22 %
Ht	4	6	10	20 %
Congenital	3	1	4	10 %
Obstructive Neph.	1	2	3	6 %
Post chemotherapy	1	1	2	4 %
TB. UTI	1	1	2	4 %
Nephrotic Syndrome	1	1	2	4 %
Neurogenic	1	0	1	2 %
Tumour lysis Syndrome	1	0	1	2 %
Total			50	100 %

Table (5) Distribution of patients for K/ DOQI targets achieved (P= 0.05)

Patients groups	Target for Ca	Target for Po4	Target for Ca byPo4	Target for PTH
Anuric patients	30 %	25 %	60 %	0 %
Preserved RRF	30 %	43.3 %	76.7 %	16.7 %
P. value =	0.75	0.18	0.303	0.14

Table (6) Hemodialysis adequacy for all patient groups, the ,mean SPKT/ v

Patients group	Count	Mean SPKT/ v	P value*
Loss RRF	20	0.91 ±0.25	0.0472
Preserved RRF	30	0.78 ± 0.20	
Total	50	0.8310.22	

* There was a statistically significant difference in Hemodialysis adequacy.

Discussion

There are several studies that demonstrate the importance of preservation of RRF for improvement of Po₄, Ca, Ca by Po₄ product and PTH metabolism (12, 15, 17) to HD patients.

In our study there was a difference although statistically not significant when we compared the two groups of patient, were those patients with preserved RRF had lower S. Po₄ levels than anuric group mean = $6.1 \pm (1.85)$ vs. $6.8 \pm (2.0)$ with 43.3% vs 25% achievement of K/ DOQI guidelines, P value = 0.18. They had higher S. Ca, mean = $7.6 \pm (1.39)$ vs. $7.4 (1.4)$ and 30% vs. 30% achievement of guidelines, P value = 0.75.

There was lower Ca by Po₄ product, mean = $45.8 \pm (13.62)$ vs. $51.1 (19.3)$ with 76.7% vs. 60% achievement of the guidelines, P value = 0.304. Mean S. PTH level was lower = $239 \pm (209)$ vs. $292 \pm (231)$ with 16.7 % vs. zero achievement of guidelines, P = 0.14. For these reasons we agree with all measures that approved to be protective for RRF such as:

Blood pressure control (8).

Avoidance of hypotensive episodes during HD (8).

The use of biocompatible membrane (12). Avoidance of nephrotoxic agents (11). Control of proteinuria (8), Dialysis water purity (12).

In addition inadequate HD can reduce the importance of RRF as a preserved RRF group had lower spkt/v than anuric group (table 6) which could be attributed to the patients compliance with HD program (21), because the clearance depend on HD and RRF clearance.

Conclusion: RRF was associated with improved Po₄, Ca, Ca by Po₄ product and PTH in HD patients.

Recommendations: A practice of all measures that can preserve RRF in maintenance HD patients was recommended .

Ethical Clearances : The consent of the patient has been taken in the hospital .

Conflict of Interest : Non

Source of Funding : Self

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Thyroid Function Test Abnormality in Patients Using Sodium Valproate

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Abstract

Background: Thyroid gland is one of most important gland in the body, function mainly through synthesis of thyroid hormones. The gland influences almost all of the metabolic processes in the body. Epilepsy is a complicated condition that may affect different areas of human life. Epileptic seizures change the endocrine environment mainly through the action on the hypothalamic-pituitary axis. The gland function could be affected by many disorders and drugs. **Objectives:** To assess thyroid function test abnormality in patients using Sodium valproate. **Patient and Method:** A case – control study conducted at Al Yarmouk teaching hospital for 50 patients with generalized epilepsy who attend neurological clinic from January 2015 to November 2015. From each a detailed history and examination had been taken age, sex, body mass index, duration of treatment and dose of each one. Inclusion criteria: patients presented with idiopathic generalized epilepsy receiving sodium valproate tablet twice daily with different total dose without any other drugs. Patient with disease other than epilepsy is excluded. Also 50 controls have no any chronic disease [hypertension, diabetes, kidney disease or drugs affecting thyroid or history of thyroid surgery, no smoking or alcoholic]. Blood samples were sent for TSH, T3, T4, f T3, f T4 in Al Yarmouk teaching hospital laboratory to assess thyroid function abnormality in these patients and compared with the general population. **Finding :** 50 patients with generalized epilepsy receiving sodium valproate who complete study 26 were female and 24 were male, their ages were ranged between 14 and 51 year old, subclinical hypothyroidism were seen in [10 / 50] 20% of patients and [3/50] 6% of control in this study . The comparison with the control group showed significant statistical difference. **Conclusion:** The percentage of subclinical hypothyroidism is significant in patient using Sodium valproate.

Keywords: Thyroid function test, Sodium Valproate, Subclinical hypothyroidism

Introduction

Thyroid hormones help all organs work well. they control body uses of food for energy .Thyroid hormones affect metabolism rate, which means how fast or slow brain, heart , muscles, liver , and other parts of body work .If body works too fast or too slowly, for example, if don't have enough thyroid hormone , might feel tired and cold. Or, if have too much thyroid hormone, might feel nervous, jumpy, and warm ⁽¹⁾.

Seizures are the hallmark symptom of epilepsy. An epileptic seizure is __a transient occurrence of signs and/ or symptoms due to abnormal excessive or synchronous neuronal activity in the brain ⁽²⁾.

The incidence of epilepsy is in the region of 80 cases per 100,000 persons per year, with different studies showing rates varying mostly between 50 and 120 per 100,000 per year. Its point prevalence has been found to be 4–10 cases per 1000 persons in most studies in united kingdom, although higher figures are found from settings in resource poor countries ⁽³⁾.

The treatment of epilepsy of all types can be divided into four parts: the use of antiepileptic drugs, the surgical excision of epileptic foci and other surgical measures, the removal of causative and precipitating factors, and the regulation of physical and mental activity. The goal of drug treatment is to create a seizure-free state if possible and with the fewest side effects ⁽²⁾.

Valproate, a pentanoic acid, is a drug for initial treatment of idiopathic generalized epilepsy with absences and juvenile myoclonic epilepsy and for generalized tonic clonic seizures. It is a broad-spectrum antiepileptic drug and is also efficacious against partial seizures. Common side effects include tremor, behavioural disturbances, weight gain, thrombocytopenia, menstrual disturbances, ankle swelling and usually minor loss of hair⁽³⁾.

Valproate (VPA) medication is associated with metabolic and endocrine changes in women with epilepsy, reproductive endocrine disorders characterized by menstrual disorders, polycystic ovaries, and hyperandrogenism are common in women taking VPA⁽⁴⁻⁶⁾.

It is known that the traditionally used antiepileptic drugs may affect thyroid function test^(7,8)

Both normal or elevated serum levels of thyroid hormones and TSH have been reported among patients taking valproate^(9,10)

Valproic acid could increase serum TSH by affecting the complex central neuroendocrine control of TSH release that in turn might elevate serum FT4⁽¹¹⁾

Neurologists now have many more antiepileptic drug (AED) options for the care of patients with epilepsy than ever before; however, choosing among the various AEDs and navigating their side-effect profiles and drug interactions can be challenging⁽¹²⁾. Therefore the aim of study to assess the effect of Sodium Valproate on thyroid function test in epileptic patients.

Patients and Method

Study Type: A case – control study conducted at Al Yarmouk teaching hospital for 50 patients with generalized epilepsy who attend neurological clinic from January 2015 to November 2015.

From each a detailed history and examination by neurologist had been taken age, sex, BMI, duration of treatment and dose. Inclusion criteria: patients presented with generalized epilepsy receiving sodium valproate tablet dose 200mg twice daily with different total dose without any other drugs, patient with other than epilepsy is excluded.

Also 50 controls have no any chronic disease [hypertension, diabetes, kidney disease or drugs affecting thyroid or history of thyroid surgery]. Blood samples were sent for TSH, T3, T4, fT3, fT4. In Al Yarmouk teaching hospital laboratory to assess the functional abnormality in these patients was compared with the general population.

Age [14- 51 years].

Statistical analysis

Each patient assigned a serial identification number. The data were analyzed using Statistical Package for Social Sciences (SPSS) version 20.

- The continuous data were represented by mean, standard deviation and range.
- The categorical data presented as frequency and percentage tables.
- The Chi square was used to assess the association between variables.
- Analysis of variances (ANOVA), and student's t-test, were used to compare mean values of thyroid function test between study groups

P – Value less than 0.05 was used as the alpha level of significance.

Finding

The study had included 100 persons, 50 controls and 50 patients with generalized epilepsy using sodium valproate.

About 52 (52%) were female and 48 (48%) were male. The age range (14 – 51) years with mean age of total 26.8±10.7.

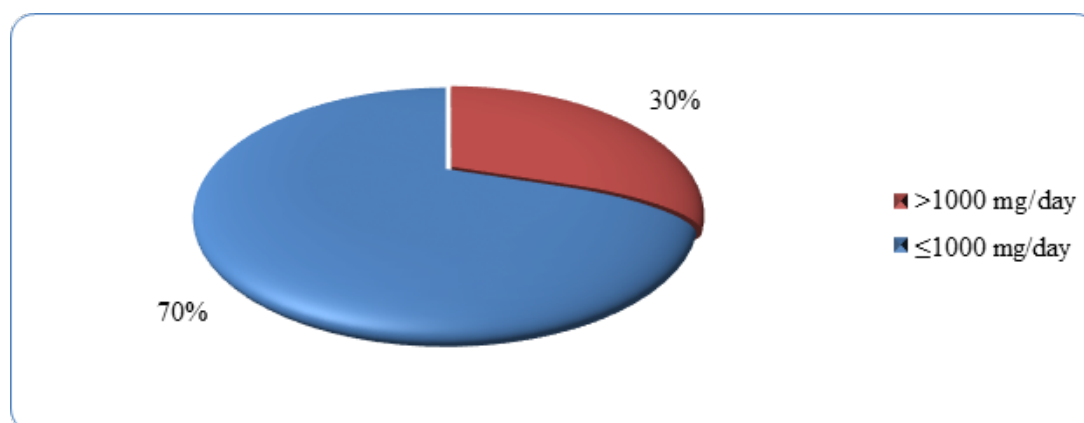
The sample were generally overweight with 64 (64%) have BMI ≥25 (kg/m²) and 36 (36%) have <25 (kg/m²) as shown in table 1.

Table 1: Demographic features of the study groups.

Variables	Cases No. (%)	Controls No. (%)	Total No. (%)	p value
Gender				
Male	24 (48%)	24 (48%)	48 (48%)	1
Females	26 (52%)	26 (52%)	52 (52%)	
Age groups				
<20 years	21 (42%)	22 (44%)	43 (43%)	
20 - 40 years	14 (28%)	11 (22%)	25 (25%)	
≥40 years	15 (30%)	17 (34%)	32 (32%)	
Age (years) ^a Mean±SD	26.5±11.2	27.1±10.1	26.8±10.7	0.776
BMI				
<25 (kg/m ²)	17 (34%)	19 (38%)	36 (36%)	0.678
≥25 (kg/m ²)	33 (66%)	31 (62%)	64 (64%)	
Total	50 (100%)	50 (100%)	100 (100%)	
Chi-square test, ^a Student t-test, SD= standard deviation				

Age range (14 – 59) years

Regarding the dose 35 out of 50 patients (70%) of patient using dose less than 1000 mg/ day and 15 out of 50 patients (30%) using dose higher than 1000mg /day. As shown in figure 1.

**Figure 1: Percentage of the cases group, according to the daily dose of sodium Valeproate, N=50.**

Regarding the mean value for thyroid function test parameters showing that TSH increased value among sample with significant p value <0.007 .

Among other parameters T4, fT4, T3 showing no significance difference between patients and control as shown in table 2.

Table 2: Comparison of mean values for thyroid function test parameters between control and cases on Valproate according to dose, =100

Parameters	Control N=50	Dose ≤ 1000 mg/day N=35	Dose >1000 mg/day N=15	p value
TSH (mIU/l)	2.9 \pm 0.7	4.2 \pm 0.9	4.4 \pm 1.2	0.007*
T4 (nmol/l)	92.3 \pm 14.7	87.5 \pm 16.3	89.9 \pm 17.6	0.385
fT4 (pmol/l)	16.7 \pm 3.1	17.4 \pm 2.3	17.9 \pm 2.3	0.255
T3 (nmol/l)	1.7 \pm 0.5	1.7 \pm 0.3	1.7 \pm 0.6	0.515

ANOVA test, all values in Mean \pm 1 standard deviation, * Significant at 0.05 level

Regarding comparison of TSH among control and patients divided according to dose below or above 1000 mg/day. This showing higher increase in TSH values above dose 1000 mg/day more than those below 1000 mg/day and both are more than control, as shown in figure 2.

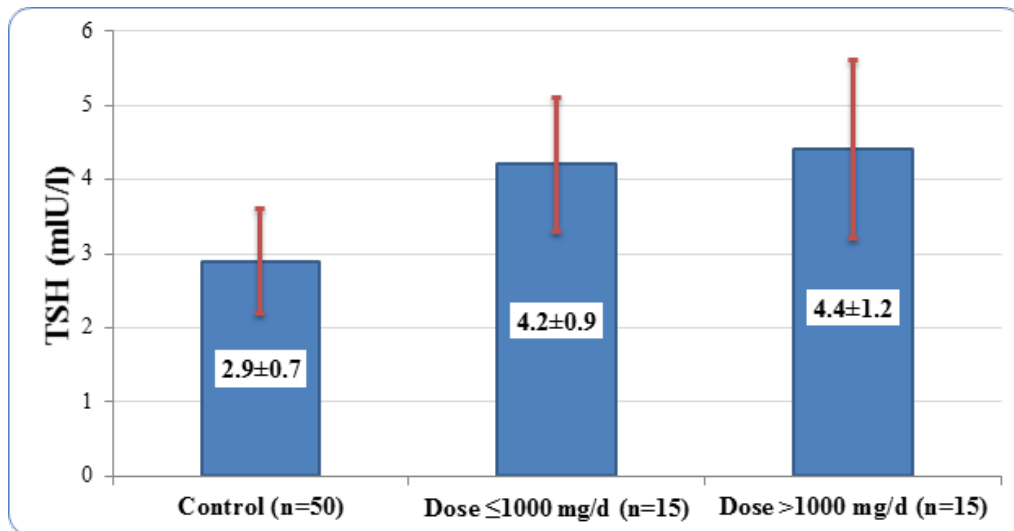


Figure 2: Comparison of TSH mean values between study groups, N=100.

Our study showing that the prevalence of subclinical hypothyroidism is 13% which is significant at 0.05 level among sample where as eutyroid is 87% as shown in table 3.

Table 3: Relation between thyroid function and Valproate treatment or dosage, N=100.

Thyroid function	Control	Dose ≤1000 mg/day	Dose >1000 mg/day	Total
Sub-clinical hypothyroid	3 (6%)	5 (14.3%)	5 (33.3%)	13 (13%)
Euthyroid	47 (94%)	30 (85.7%)	10 (66.7%)	87 (87%)
Total	50 (100%)	35 (100%)	15 (100%)	100 (100%)

The percentage of subclinical hypothyroid in study sample 13% .

The percentage of sub -clinical hypothyroid is 20% in patient treated with

Valoprate.

Discussion

A number of studies examined links between valproate and thyroid hormones, but in our society, there were little data about this, Each AED has unique characteristics , including spectrum of activity, cost, Pharmacokinetic and pharmacodynamic properties , likelihood for dose related side effects , and risk of serious health problems, such as idiosyncratic reactions ⁽¹³⁾.

This study investigate effect of valproate on thyroid function in patients age range from [14 - 51 years] ,however most of previous studies either studies effect in children like L.k . Vainionpaa et al (14) in which age rang [8.4-18.5years] or in adults like Aksoy et al ⁽¹⁵⁾ for patient age [18-40 years].

Its known that the traditionally used antiepileptic drugs may affect thyroid function test ⁽⁶⁻⁸⁾.

Valproic acid could increase serum TSH by affecting the complex central neuroendocrine control of TSH release that in turn might elevate serum FT4⁽¹⁶⁾

The result of this study showing increase frequency of subclinical hypothyroidism in 20% of treated patient receiving valproate that consistent with study of Yehia, et al⁽¹⁷⁾ that demonstrate significant increase of TSH in 20% of patient using Valproic acid .

Also consistent with study of U.Yilmaz et al ⁽¹⁸⁾ conducted on children and adolescent epileptic patients

receveing Valproic acid indicate that frequency of Sub-clinical hypothyroid was 28%.

Sub-clinical hypothyroid with mild to moderate elevated TSH which has been demonstrated in some Studies ⁽¹⁴⁾, but not all studies ⁽¹⁹⁾.

Also our result show increase in sub-clinical hypothyroid with increase mean of duration of treatment [13±3.7] with p-value 0.027 that consistent with study of ⁽²⁰⁾ that study risk factor for development of sub-clinical hypothyroid found that increase duration of treatment between 6-24 months associated increase risk of development of sub-clinical hypothyroid.

Both normal or elevated serum levels of thyroid hormones and TSH have been reported among patients taking valproate ^(21,22).

Also in our study regarding the high dose >1000 mg/day for 5 patient out of 15 (33.3%) associated with more increase in sub-clinical hypothyroid, that found also in study of Sherifa A, et al ⁽²⁰⁾ increase dose and serum level are risk factor for sub-clinical hypothyroid with valproate dose 1400 ± 250.

On the other hand , this result is in disagreement with previous study By Isojarvi *et al.*,⁽¹⁹⁾ who studied thyroid function in men taking antiepileptic drugs (carbamazepine and valproate) and found a decrease in T4 level, but there is no alteration in TSH and T3 levels.

Regarding other TFT parameters which show no significant difference that consistent with De Vries *et al.*⁽²³⁾, that showing higher level of TSH and lower level of FT4 than did untreated group, although still within normal ranges .other study done by SahuJk et al⁽²⁴⁾, which found FT4 were found to be unchanged

while TSH level increased.

Conclusions

In light of the result of the this study,

1) We conclude; the incidence of the sub-clinical hypothyroid is higher among patients with epilepsy using valproate compare with the population.

2) Increase duration of treatment increasing risk of development of sub-clinical hypothyroid.

3) Increase dose of valproate increasing risk of development of sub-clinical hypothyroid.

Recommendations:

1. To conduct further larger study in the future with wide sample size.

2. Encourage routine TFT in follow up patients with epilepsy using valproate.

Limitation:

1. The duration of the conducted study was only 10 months.

2. Small sample size of the thesis which include 50 patients

3. The paucity of similar study to compare the data with it.

4. The conducted study includes patients for one hospital only (Al yarmouk teaching hospital).

Ethical Clearances :The consent of the patient has been taken in the hospital .

Conflict of Interest : Non

Source of Funding : Self

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Study the Correlation between the Period Response of Iraqi Patients and Therapeutic Regime (Oral Triamcinolone Only Compared with used both Systemic Dapsone and Triamcinolone) In minor Aphthous Ulcer (MAU)

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Background : recurrent aphthous stomatitis (RAS) is a common medical problem, Which affect all ages through the world , and it includes all economic state. therefore aim of current study is determine the effect of systemic dapsone in treatment of minor aphthous ulcer (MAU). **pateints and method;** case study of (93) pateints with minor aphthous ulcer (MAU) attended to sulabddin general hospital , that divided into two groups : as group (A) fifty-two patients treated with oral triamcinolone alone. group (B) forty –one patients treated with oral triamcinolone plus systemic dapsone. **Results :** minor aphthous ulcer (MAU) mainly affect young age (3-15) years , with female predominance , poor person more lible to this problem. there s significant improvement in time of healing and pain reduced, in group (B) .

Keyword : *Therapeutic ; oral triamcinolone paste ; systemic dapsone ; minor aphthous ulcer (MAU) .*

Introduction

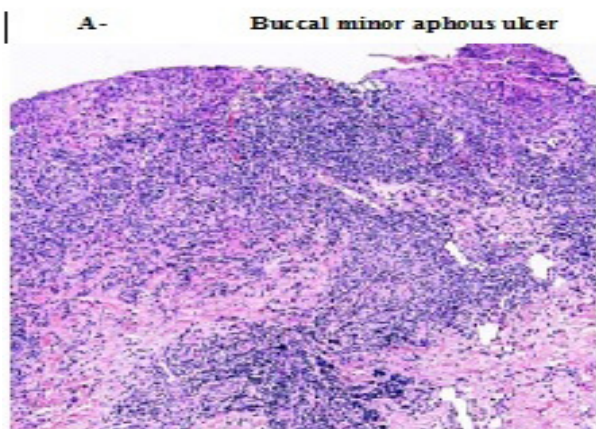
Aphthous ulcers are break in mucosa of the mouth or lips , it's the most common oral mucosal lesion, but the etiological factors are unknown. aphthous ulcers in most person, are recurrent and often resolved from (1-3) weeks ^(1,2). There is no specific treatment that ensuring no recurrence of ulcer , the previous studiesshowed that any agent(excluded the anti-inflammatory) , can reduce the severity and recurrence of RAS(3). therefor the patients must know that the most cases of these types of ulcers will resolve without any therapy ^(1,2).

Three clinical types of RAS can be classified that includes: herpetiform ulceration; major RAS and Minor RAS. But the most common type is the Minor aphthous ulcer which is about (85%) of all patients have oral ulcer lesion ⁽⁴⁾ and the family history presented in (40%) of cases. so the Minor aphthous ulcer (MAU) are usually located in the anterior part of the oral cavity, other parts of mouth included (the buccal , labial mucosa and floor of mouth and the lateral and ventral, aspect of tongue), (5 mm- 1cm) in size ⁽⁵⁾(fig.1), firstly appears in childhood and adolescence , compare to Major aphthous ulcer , its less common in the childhood and adolescence , and the size of this ulcer is about (1-3) cm, usually deep with presence of scar after healing, and healing usually within

3 weeks, as well as the herpetiform aphthous , which is much less (1-2) mm and usually (10-100) at the same time , it take time to heal without scar after healing , and firstly appear in young adults , the Diagnosis of aphthous stomatitis depend on clinical examination, laboratory investigations usually unimportant or unnecessary, despite some diagnostic testing might be an important in recurrent ; or persistent cases ^(6,7).

Pateints and Methods

The case study of (93) pateints with minor aphthous ulcer (MAU) who attended to sulabddin general hospital during the period (September 2016 to April 2018) . All cases were diagnosed by the specialist doctor and divided into two groups : as group (A) fifty-two patients treated with oral triamcinolone alone. group (B) forty –one patients treated with oral triamcinolone plus systemic dapsone, dapsone given in small doses and short period, (25mg) daily for 3 days , then (50mg) daily for 3 days . blood count, Retic count, LFT, RFT was done for group (B) before they received dapsone . data of the patients includes (name, age, gender, and economic state) was taken by questionnaire .



A- Buccal minor aphthous ulcer
C-Histopathology of Buccal minor aphthous ulcer
(eroded surface) epithelium –inflammatory cells



B-Buccal minor aphthous ulcer during healing
D- Tongue minor aphthous ulcer

Finding

Table (1): Frequency of patients who have minor aphthous ulcer (MAU)

According to age groups

Age groups (years)	No.	%
≤ 15	32	34.4
16-25	18	19.35
26-35	16	17.2
36-45	13	13.97
46- 60	14	15.05
Total	93	100

Results in table (1) showed that the most common age group with minor aphthous ulcer (MAU) (3-15) years as (No.=32, 34.4%) , followed in age group(16-25) years as (No. = 18, 19.35%).

Table (2): frequency of patients who have minor aphthous ulcer (MAU) according to gender

Gender	No.	%
Male	35	37.63
Female	58	62.37
Total	93	100

In table (2) founded the female who have minor aphthous ulcer (MAU) (No. = 58, 62.37%), more than male (No. = 35, 37.63%).

Table (3): Distribution of patients who have minor aphthous ulcer (MAU) according to economic level

economic level	No.	%
Poor	37	39.8
Middle	29	31.2
Good	21	22.5
Very good	6	6.5
Total	93	100

Results in table (3) showed that poor patients more suffering from recurrent aphthous ulcer as (No. = 37, 39.8%) compared to good and very good economic state as (No. = 21, 22.5%) and (6, 6.5%) respectively.

Groups	Period of patient response to treatment			
	Within 5 days		After 5 days	
	NO.	%	NO.	%
A	19	36.5	33	63.5
B	31	75.6	10	24.4
Total	52	100	41	100
P.value	0.05			

Note: groups A who received triamcinolone alone; Group B who received triamcinolone+ systemic dapsone.

Results in table (4); showed significant improvement in period of healing of aphthous ulcer in group (B): that treated with oral triamcinolone + systemic dapsone. (No. = 31, percentage 75.6%, within 5 days) and (No. = 10, percentage 24.4% after 5 days). While in those who treated with triamcinolone alone, group (A): (No. = 19, percentage 36.5%, within 5 days) and (No. = 33, percentage 63.5% after 5 days) (p.value = 0.05).

Discussion

Current study showed that the most common age group involved with minor aphthous ulcer (MAU) (3-15) years as, followed in age group (16-25) years as (No. = 18, 19.35%), but increase Aphthous ulcers usually with increased age and the MAU included in about 80% of total suffered oral ulcer, oral ulcer presented in (5-66%) among different nations⁽⁸⁾.

The prevalence of MAU in the kids of US about (40%)⁽⁹⁾. but in Iran it is about (25.2%)⁽¹⁰⁾. Female who have minor aphthous ulcer (MAU) was more predominant, this results are entirely consistent with Santosh *et al.*, 2014 who showed that Females more affected with oral ulcer (56.3%) than males (43.7%)⁽¹¹⁾, and McCullo *et al.* 2017 showed that female patients are more likely affected than males, which may be related to dysmenorrhea, menstrual cycle and pregnancy⁽¹²⁾, as well as sex hormones (steroid) that have an active role in RAS disease⁽¹³⁾, as well as the drugs, food hypersensitivity, oral trauma, nutritional deficiency (vitamins, iron and folic acid) and emotional stress.

Also these study showed that, the poor patients more

suffering from recurrent aphthous ulcer, compared to good and very good economic state, but Sunday *et al.*, showed that aphthous ulcer are common medical problem with high prevalence on the high socioeconomic state, it is more common in twenties male, with family history in more than one third of the affected patients⁽⁴⁾.

There's significant improvement in healing time when we used combination of oral triamcinolone plus systemic dapsone in compare with use of oral triamcinolone alone. Management of recurrent aphthous stomatitis remain great challenge. Local steroid indicated in small and mild ulcer^(14; 15).

Triamcinolone 0.1% is effective in decreasing pain and reduce the healing time of recurrent aphthous ulcer⁽¹⁶⁾, so triamcinolone acetonide in Orabase improve healing and reduce pain of recurrent aphthous ulcer⁽¹⁷⁾. The systemic drugs is very effective in treatment of recurrent aphthous ulcer, (corticosteroid and immunosuppressor) like pentoxifylline, colchicine, dapsone and thalidomide.

Dapsone used mainly for treatment of leprosy at the dose of (50) mg⁽¹⁸⁾. It used for treatment of large and resistant recurrent aphthous ulcer in dose of (25mg) for 3 days; (50mg) for 3 days, (75mg) for 3 days, and (100mg) maintenance⁽¹⁹⁾. Also Dapsone was effective when used with colchicine in treatment of severe recurrent aphthous ulcer⁽¹⁸⁾.

Recommendation

1- Future study about the immunological process that leading to appearance of recurrent aphthous ulcer.

2- Dapsone has important role in treatment of recurrent aphthous ulcer mainly if its combined with other local treatment like Triamcinolone

Conclusion

There's significant improvement in healing time and pain reduce when we added the systemic dapsone to regime of treatments.

Ethical Clearance: from research ethic committee in Tikrit university/college of medicine

Source of Funding : Self

Conflict of Interest : None

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Color Changes in Dry Pigmented Tech-Sil25 Maxillofacial Elastomer after Artificial Weathering

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Abstract

Background : Maxillofacial prosthesis that processed from silicone elastomer materials have their own physical properties that will lead to degradation on frequent subjection to ultra violet sunlight that will need to replacement of the prosthesis because of its pigments undergo color changes.

Aim of the Study : The objective of this study is to evaluate effect of three periods of artificial aging at (75h, 150h, 300h) on color changes of room temperature vulcanized (Tech-sil25) silicone maxillofacial material after incorporation 0.2 % by weight of dry pigment (Burnt sienna) by using spectrophotometer test. **Materials and Method** Total of 20 circular disk (2mm thick and 20mm diameter) specimens were fabricated by incorporation of 0.2 % by weight of Burnt sienna to room temperature vulcanized (Tech-Sil25) silicon divided into two groups control and study group, 10 samples for each group. The control group samples were tested for color change with spectrophotometer and the study group were subjected to artificial weathering ultraviolet for (75, 150, and 300 h) then after each period they tested for color change with spectrophotometer. All data were analyzed with a descriptive statistical analysis, one-way ANOVA test, LSD test. **Result:** Subjection of the burnt sienna pigmented (Tech-sil25) maxillofacial silicone elastomers to ultraviolet light resulted in a highly significant increase ($P \leq 0.01$) in mean values of color absorbance after artificial weathering for (150-300 hours) where there is no significant differences in mean values of color absorbance after artificial weathering for (75 hours). **Conclusion:** The color change in silicone elastomer pigmented with burnt sienna is increase with increasing the aging period.

Keywords : Color changes ; dry pigmented ; Tech-Sil25 maxillofacial ; artificial weathering.

Introduction

Restoring of the patient esthetic and protecting the remaining tissue is the main goals in maxillofacial prosthodontics which will positively improve the patient self-esteem and restore patient's normal life as possible. ⁽¹⁾

Maxillofacial prosthetic materials elastomer that used for the processing of maxillofacial prostheses has private mechanical and physical properties. Unfortunately, maxillofacial materials have a low long-term durability, and a restoration may become ruptured or lose its primary color within a short interval. The overall degradation is a result from certain environmental conditions, such as 1-exposure to ultra-violet (UV) light of natural sunlight, 2- drying and wetting of the elastomer, 3- abrasion of external surface because

of application and removal of some cosmetics. This involves exacting replication of subdermal, commonly referred to as intrinsic coloration and external or extrinsic coloration. The color alteration of maxillofacial prosthesis may be caused by change of extrinsic or intrinsic pigmentation resulting from external environmental conditions also it can relate to the color stability of elastomer itself. ⁽²⁾

The volume, shape, position, transparency and texture of the patient must be taken in consideration when processing the maxillofacial prostheses. Although when the technicians are choosing the best maxillofacial materials to manufacture the prostheses also they have difficulty in mimicking the color of patients' normal skins which lead to some color alteration. ⁽³⁾

Materials and Method

Total of 20 circular disk (2mm thick and 20mm diameter) specimens were fabricated by incorporation of 0.2 % by weight of Burnt sienna to room temperature vulcanized (Tech-Sil25) silicon divided into two groups control and study group, 10 samples for each group.

The samples dimensions were designed by using Auto CAD 2013 then it processed by using the CNC milling machine to make the mold which will be used for silicone pouring ⁽⁴⁾

The specimens were fabricated by using the vacuum mixer, in which 0.2 % by wt. (0.12 g) of burnt sienna pigment was added carefully and weighted several times to insure the exact pigment percentage. After weighing the proper amount of pigment the silicone base was

added at 59.88 g and mixed by vacuum mixer for 7 minutes at speed 360 rpm under vacuum -10bar. For better incorporation of pigments into silicone, pigments was mixed with the base for 2 minutes by mechanical mixer without vacuum then mixing under vacuum for 5 minutes \pm 5 seconds by the mechanical mixer according other studies. Then a drops of catalyst were add by dispensing in many areas and not in center only, to aid in gaining homogeneity rapidly while the glass beaker was on the digital scale ⁽⁵⁾

Before closing the mold, the mold covers was painted with petroleum jelly and hex nuts are prepared and screws are inserted in each hole at the corner of the mold. Then silicone mixture is poured gradually. When pouring was finished, the air bubbles were removed (Figure 1).

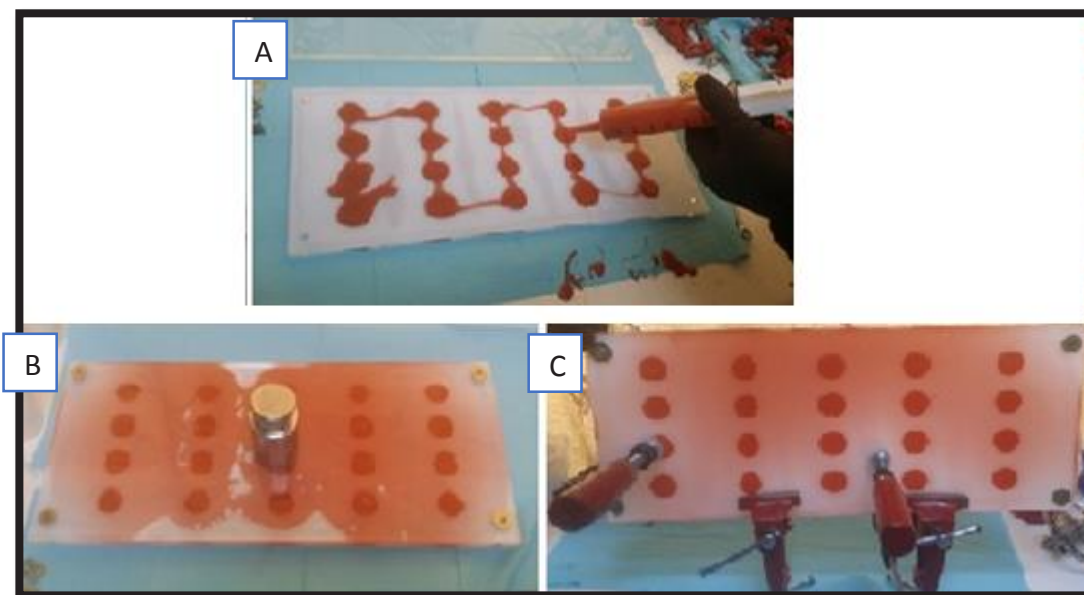


Figure (1) A, pigmented silicone was gradually poured then air bubbles was removed B, 1 kg weight is applied over the cover C, clamps are applied and tightened over the four mold sides

Needle was used to blowing-up air bubbles on the surface. When all air bubbles were broken-up, the mold was covered, that was previously painted by the petroleum jelly over the matrix by using tissue papers. ⁽⁶⁾

Covering the mold was poured from one side to another laying the lower edge of cover while holding carefully the upper edge, then the cover was lowered slowly and carefully onto matrix to push out any excess of silicone material and air bubble out of it for burnt sienna. Then, the weight of 1 kg was added on the mold

center then the cover was carefully tightened by nuts and screws at the corners of the mold then four clamps was applied at the middle and sides (Figure 1). After that the closed mold were storing away that avoiding the light at ($23^{\circ}\text{C} \pm 1$) for about 24 hours to complete RTV silicone material vulcanization according to manufacturer's instruction. All the samples were removed from their molds after setting completion of the silicone. Care was taken to avoid straining the samples during demolding process. The excess flashes then eliminated by using a scalpel and surgical blade #10. The samples were subjected to aging in Weather-Ometer machine

(QUV) model (Xenon Arc Ci4000, Atlas Material Testing Technology, USA) according to a most popular accelerating standardization (ASTMG154) under cycle 7 aUV Testing Lab. The mentioned cycle is exposing the samples to a cycles of 5 hours of UV source of light at 340nm with a high temperature of 60°C, then followed by 4 hours (spray for 0:15 minutes) and condensation (dew) at 50°C for 3:45 minutes at intervals of 75, 150 and 300 h. The samples for all groups were labeled with a permanent marker to account and distinguishing the samples after weathering. All the samples were kept in a dark container because the samples exposed to receive a light that effect on the result of color measurement of the samples, each sample was measured with the light absorbance using a spectrophotometer (UV Visible

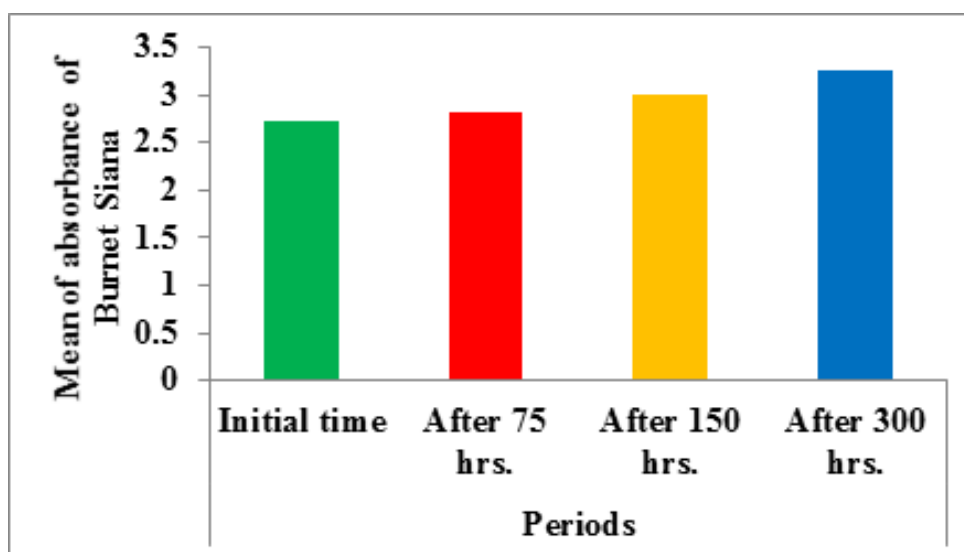
1800 spectrophotometer, Shimadzu Co., Ltd., Japan) before and after ageing.⁽⁷⁾

Finding

Table (1) and figure (2) represents a statistics such as mean parameter values, standard deviation and standard error a 95% confidence interval of mean parameter value in the studied population, and a two extreme values (minimum and maximum) of the absorbance measured of readings, distributed according to treated groups with Burnet sienna along different periods of experimental times (Initial time, After 75 hrs., After 150 hrs., and After 300 hrs.), where the lowest mean at the initial time was (2.733) and the highest mean at after 300h period of time was (3.262).

Table (1): Statistics concerning Burnet sienna pigment absorbance distributed in different periods.

Absorbance							
Groups	Periods	No.	Mean	Std. Deviation	Std. Error	Range	
						Min.	Max.
Burnet Siana	Initial time	10	2.733	0.122	0.038	2.563	2.944
	After 75 hrs.	10	2.809	0.093	0.029	2.677	2.939
	After 150 hrs.	10	3.009	0.249	0.079	2.808	3.680
	After 300 hrs.	10	3.262	0.443	0.140	2.838	3.998
	Total	40					



Figures (2): Statistics concerning Burnet sienna pigment absorbance distributed using a bar chart plotting in different periods.

In table (2) statistically show the test of equal variances assumed for Burnet siennapigment absorbance, as well as equal mean values assumed through applying "Levene and one-way ANOVA" respectively. The results show a high significant of differences are account at $P < 0.01$ in at least with one pair of studied treatments regarding different periods of times

Table (2): Testing equal variances and equal mean values for absorbance of Burnet sienna along different periods of times.

Absorbance				
Burnet Siana	Testing Homogeneity of Variances		ANOVA- Testing Equality of Means	
	Levene Statistic	P-value	F-test	P-value
	8.503	0.00 (HS)	7.927	0.00 (HS)

HS: Highly sig. at $P < 0.01$

Table (3): Probable Pairs wise comparisons using (LSD) test among Burnet sienna pigment absorbance along different periods of times.

Burnet sienna (absorbance)		LSD test	
Periods		P-value	C.S.
Initial time	After 75 hrs.	0.523	NS
	After 150 hrs.	0.026	S
	After 300 hrs.	0.00	HS
After 75 hrs.	After 150 hrs.	0.101	NS
	After 300 hrs.	0.001	HS
After 150 hrs.	After 300 hrs.	0.040	S

HS= Highly sig. at ($P < 0.01$); S= Sig. at ($P > 0.05$)

& NS= Non Sig. at ($P > 0.05$).

Statistically In table (3) the (LSD) test results shows that there are no significant differences between the initial period and aging after 75hrs, while there is a significant different was accounted between the initial time and after 150hrs, while there is a high significant different was accounted between the initial time and after 300hrs, also there is no significant different was accounted between aging after 75hrs and aging after 150hrs, while there is a high significant different was accounted between aging after 75hrs and aging after 300hrs, finally there is significant different was accounted between aging after 150hrs and aging after 300hrs.

Discussion

General degradation of polymers are mainly result from the environmental factors aging that effect on the silicone elastomer, which can cause changes in the color itself. In several cases it will cause color changes of the pigments, changes in chemical and physical characteristics can occur from the polymers weathering, which can cause alteration in their properties. The mainly cause of deterioration for polymeric materials is the combined action of sunlight and oxygen, photo-oxidative attack which effect their chemical structure.⁽⁸⁾

Other factors that associated with color change, such as, surface roughness accumulation

between the studies; researchers had recognized, that there is a level of differences in the length and type exposing, testing method, concentration and addition of pigments or opacifiers and type of maxillofacial silicone.⁽¹⁴⁾

The non-significant alteration in color absorption may be a consequence of mechanical and chemical activation (wiping the specimens before storage) that may probably washed away of some particles of pigment that will accumulated on the silicone material surface during the storage.⁽⁶⁾

The presence study is concluded that the absorbance measurement has increasing of score of burnt sienna study group when compared with control group. Temperature, light, and mechanical force are the primary factors affecting the quality of service of silicone elastomer materials. According to the results that obtained in this study, color change is occurs, irrespective of the accelerating aging period. That agree with Villalta et al., 2006 that state it is known that both extrinsic and intrinsic factors may be caused a chromatic alteration.⁽¹⁵⁾

It is also agree with Gary and Smith, 1998 indicated that early color changes in maxillofacial silicone prosthesis may be the result from the degradation of the prostheses by UV-light to a susceptible pigments, whereas long-term color alteration also cause by the color changes that occur within elastomers itself.⁽¹⁶⁾

As well agree with Lekha, 2015 indicated that pigmented silicone material with burnt sienna revealed a more color alteration when exposed to aging other than pigmented silicone material with cobalt blue.⁽¹⁷⁾

The presence study is disagree with Kiat-amnuay et al., 2009 who stated that spectrophotometer test for Burnt sienna and other pigment groups and the results when (compared with the (control group show a color change principles at each interval will increase significantly and the Burnet sienna is remain its primary shade over time.⁽¹⁸⁾

Conclusion

Within the limitations of this study, the following conclusions were achieved: color change is increase with increasing the aging period silicone elastomer pigmented with burnt sienna.

Conflict of Interest: None

of (stains, (dehydration, water (absorption, infiltration, degradation from use, chemical (degradation and oxidation (during double carbon-reactions to produce the peroxide (compounds, all can cause degradation of pigment.⁽⁹⁾

The most important character that used by patients to evaluate the facial prostheses is color. Change in color is mainly caused by aging process, adding additives or use of disinfectants. The artificial accelerated weathering are produced a changes greater than the outdoor weathering or normal prosthesis use.⁽¹⁰⁾

Therefore ASTM G-154 cycle 7 with a high temperature 50-60 degree and a high irradiation 1.55W/m² for 75 hours, 150 hours and 300 hours was chose in this study.

Regarding the present study, the highest mean value that obtained after 300hrs of accelerated weathering for silicone elastomers material with (burnt sienna) pigmented specimens. This could be because of the particle size of the added pigment or even because of accelerated weathering that cause a more exposure to heat and humidity which may cause more increase the color changes. Also the silicone elastomers have a low level of material energy of cohesive that will weaken the molecular particle interaction. So, small one particles will lean towards aggregation, but other larger ones will detached from polymer itself, and will not back to reinforce the silicone material.⁽¹¹⁾

A highest mean value of the color change of aging after 300hrs that obtained with burnt sienna pigmented maxillofacial silicone elastomer of spectrophotometer test than the other group. According to May and Wang 1996, the difference in color change can be caused by to the chemical properties of the silicone elastomer as well the chemical nature of pigment itself. The pigment may be get change or leach out because of accelerated weathering.⁽¹²⁾

The color alteration of Tech-Sil25 maxillofacial silicone elastomer was unacceptable when subjected to an extra oral aging.⁽⁶⁾

The discoloration of material itself can result from intrinsic factors involve with alteration in the matrix. While the absorption of substances represents the extrinsic factors, also cause discoloration.⁽¹³⁾

The results of color change levels are difference

Source of Findings: Self

Ethical Clearance: Nil

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Implication of Silver Nanoparticles to Enhance the Potential Therapeutic Activity of Doum Palm (*Hyphaene Thebaica*) Extract for *in vitro* Sperm Activation of Asthenozoospermic Infertile Men

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Abstract

Male infertility is a worldwide public health issue which affected a significant proportion of humanity. Many herbs extracts have been well documented as a therapy for infertility. This study has uniquely examined for the first time, the role of the bioactive effect of Doum Palm extract using silver nanoparticles (NPs) on improving the sperm motility of infertile human male. Semen samples of ten patients diagnosed with asthenozoospermia were collected. Semen samples were collected from ten voluntary normozoospermic males used as positive control. Doum Palm extract with and/or without silver NPs was investigated using *In vitro* sperm activation layering technique. The present study has demonstrated that there was a significant ($p < 0.05$) effect of Doum Palm extract with and without using silver NPs on active sperm motility when compared with control semen samples. Interestingly, this study has found that the application of silver NPs as a drug delivery vehicles for Doum Palm extract has a significant implication on enhancing the sperm motility *in vitro*. This finding suggested that the implication of NPs technology in assisted reproductive techniques (ARTs) may have a great future on improving sperm quality.

Key words: Male infertility, Doum Palm (*Hyphaene thebaica*), Extract, Silver nanoparticles.

Introduction

Male infertility is a global growing problem among men, may occur due to the influences of several factors including; abnormal sperm function, production of decreased sperm count, illnesses, injuries, chronic health issues and choices of lifestyle 1. In general, the quantity and quality of sperms have impact effect on man fertility. For example it is difficult for a man to cause pregnancy if his ejaculated sperm number was low or if sperm reveal poor morphological and functional quality 2.

Since the breakthrough of ARTs in 1970s, and the understanding of infertility term has dramatically changed. In particularly, the highly remarkable role of intensify the opportunities for infertile couples to have babies can be achieved by using the intracytoplasmic sperm insemination (ICSI) and the *in vitro* fertilization technique (IVF) 3,4. Despite the advantages of ARTs, the success of ARTs are not guaranteed, and patients

often have to undergo more than one cycle of harmful medication treatment before they are successful. Therefore, the application of medicinal herbs as an alternative of medicinal in ARTs could successfully increase the outcome of infertility treatment and decrease the cost 5,6,7. There are many medicinal herbs extract have been remarkably investigated for their therapeutic activity on improving sperm quality for ARTs. In fact medicinal herbs have been widely examined to boost different aspects of health care due to their bioactive contents, as they possess different biological therapeutic activities including; antimicrobial, antioxidant and anticancer effect 8,9,10,11. One of these herbs is Doum Palm (*Hyphaene Thebaica*) extract. Doum Palm is natively found in the tropical countries, particularly Upper Egypt. Doum Palm also known as gingerbread tree's edible oval fruit 12. Furthermore, the bioactive effect of Doum extract has been investigated in ARTs for successfully improving the outcome of infertility

treatment.

Several studies have suggested that Doum extract has potential effect on improving male reproductive function [3,13]. Clearly, these studies have adapted the traditional and common investigation method of combination of medicinal herbs extract with ARTs. Whereas, in current study the main objective was to boost the outcome of success rate of in vitro infertility treatment via the combination of nanotechnology with ARTs. The combination of medicinal herbs extract with ARTs using nanoparticles technology is novel and has not been well studied. Therefore, the study will implicate the silver NPs as a delivery vehicles for Doum extract for in vitro treatment of asthenozoospermic infertile men.

Materials and Method

Preparation dried extract of Doum Palm

Doum Palm fruit has been obtained from the local market. The Doum fruit was dried and grinded. 100 ml of distilled water was added to 20 gm of Doum Palm powder and refluxed for 3 hours. Once the mixture was homogenized and the powder was completely dissolved, the mixture was filtrated by using a sheet of gauze in a clean suitable flask. Finally, the filtrated solution transferred to sterile Petri dishes (Falcon, USA) and dried on an incubator at 37 °C 14.

Preparation of aqueous Doum Palm extract for in vitro sperm activation

The aqueous working solution of Doum extract was prepared by dissolving 0.5 mg of dried Doum extract with 10ml of phosphate buffer solution (PBS) (Sigma-Aldrich, USA) in plastic test tube (Falcon, USA) contained broad spectrum antibiotic. For the purpose of in vitro sperm activation, a 20% of Doum aqueous working solution has prepared by adding 8 ml of PBS to 2 ml of the aqueous working solution of Doum extract. The aqueous solution was filtered using Millipore (Millipore, USA) 0.45 µM. Media was adjusted at pH 7.5-7.8 and stored at 25 °C.

Mixture preparation of silver nitrate nanoparticles with aqueous Doum extract for in vitro sperm activation

The mixture of silver nitrate NPs with aqueous Doum extract was prepared by adding 0.58 mg of silver nitrate to 50 ml distal water. Then, the working solution

of silver nitrate nanoparticles with aqueous Doum extract for in vitro sperm activation 10 ml of aqueous Doum extract was mixed with 50 ml of the solution of silver nitrate. Later on, the final mixture of aqueous Doum extract and silver nitrate NPs kept on magnetic stirrer until the brown colour of mixture changed to the brown red colour.

Experimental design

Following the ethical approval and informed consent from all donors, semen samples were collected from ten patients diagnosed with asthenozoospermia enrolled in the Biotechnology Research Center and private Laboratory through the period from December 2018 till March 2019. Each semen sample was divided into three groups. The semen of the first men group (i.e. control group) were treated with PBS only. Semen of the second group were treated with Doum extract only (i.e. Extr. group) and semen of the third group were treated with a solution containing Doum extract and silver NPs (i.e. Extr. +NPs group). Samples were incubated for 1 hour at 37 °C in CO2 incubator. The inverted microscope was used to examine the Sperm concentration, sperm motility and morphologically (Wild Herrbrug, Switzerland) with 40X magnification. The examination was performed in each group before and following in vitro incubation using layer technique as described by Al-Dujaily et al., 15.

Statistical Analysis

GraphPad Prism 8.0.2 software was used for data analysis. The relationships between parameters and differences between groups were tested for significance using Ordinary one-way ANOVA of One-way ANOVA with Sidak's multiple comparisons test. Data have been shown as Means± standard error of the mean (M±SEM). p values of <0.05 were considered significant.

Results

The results revealed a significant improvement on concentration of sperm, number of motile sperm of grad A in both treatment groups (Doum extract only group or Doum extract with NPs group) and in morphologically normal sperm (%) when compared with non-treated group i.e. control group (Table -1)

The sperm concentration after in vitro activation was significantly (P<0.05) decreased in control group compared to pre-activation and post- activation groups.

The same observation was reported when using Doum extract medium alone. However, there was no significant differences in sperm concentration between pre-activation (60.45 ± 9.80) and post-activation when the medium containing both the Doum extract and NPs (56.0 ± 11.61).

There data showed highly significant ($p < 0.0001$) increase in the number of motile sperm of grad (A) of sperm activated in vitro in Doum extract with NPs medium compared with group treated with Doum extract only (Table-1 and Figure -1A). In addition, there was a significant increase in the number of motile sperm of grad B in both treatment groups (Doum extract only group or Doum extract with NPs) when compared with non-treated group i.e. control group; $p = 0.0025$, $p < 0.0001$, respectively (Figure B). Likewise, the data has showed a significant increase in the number of motile sperm of grad (B) in Doum extract with NPs group compared with group treated with Doum extract only $p = 0.0029$ (Figure B).

No significant differences on grad C of sperm motility in both treatment groups (Doum extract only group or Doum extract with NPs) when compared with non-treated group, $p = 0.6845$, $p = 0.4631$ respectively, (Figure C). Although there was slightly differences in the number of motile sperm of grad (C) in Doum extract with NPs group compared with group treated with Doum extract only but it was not insignificant ($p = 0.074$, Figure C).

On the other hand, the result revealed a significant elevation in the number of motile sperm of grad D in both treatment groups (Doum extract only group or Doum extract with NPs) when compared with non-treated group i.e. control group $p < 0.0001$ for both groups (Figure D).

The data of present work revealed that there was a significant ($p < 0.05$) elevation in MNS percentage post-activation compared to pre-activation. However, there was no significant ($p > 0.05$) different in the percentage of MNS post-activation using any medium as shown in table -1.

Table-1: Effect of Doum Palm extract with and without NPs on certain sperm parameters using layer technique for in vitro activation

Certain sperm parameters		Pre activation	Post activation		
			Control	T1	T2
Sperm concentration (m/ml)		$60.45 \pm 9.80a$	$40.2 \pm 10.68c$	$51.6 \pm 10.66b$	$56.0 \pm 11.61a$
Sperm Motility (%)	Active sperm motility A	$2 \pm 1.67d$	$8.1 \pm 1.85c$	$15.5 \pm 1.72b$	$26.0 \pm 6.58a$
	Active sperm motility B	$10.3 \pm 7.52d$	$15.7 \pm 2.71c$	$23.3 \pm 3.92b$	$30.8 \pm 6.23a$
	Inactive sperm motility C	$35.7 \pm 8.43b$	$27.6 \pm 2.22a$	$29.7 \pm 3.74a$	$24.8 \pm 6.73a$
	Immotile sperm D	$52 \pm 6.72d$	$48.6 \pm 4.87c$	$31.5 \pm 5.04b$	$19.4 \pm 9.13a$
Morphologically Normal sperm (%)		$40.56 \pm 8.74b$	$54.4 \pm 7.48a$	$55.7 \pm 9.8a$	$56.4 \pm 8.66a$

Values are expression as mean \pm SEM

Number of Samples = 20

T1=medium with Doum extract only. T2= medium with Doum extract and NPs

Figure 1: Silver NPs enhanced the potential therapeutic effect of Doum extract on progressive motility of sperm of asthenozoospermic male. A) Data showed both extract alone group and extr+NPs group has significant effect on sperm motility of grad (A) when compared with control $**p = 0.0011$, $****p < 0.0001$ respectively. B) Data showed both extract alone group and extr+NPs group has significant effect on sperm motility of grad (B) when compared with control $**p = 0.0025$, $****p < 0.0001$ respectively. C) Data showed both extract alone group and extr+NPs group has NO significant effect on sperm motility of grad (C) when compared with control. D) Data showed both extract alone group and extr+NPs group has significant lower effect on sperm motility of grad (D) when compared with control $****p < 0.0001$ for both groups.

Discussion

Following in vitro activation, there was a significant decrease in sperm concentration in both control samples and medium containing Doum extract only. This is due to the failure of the dead and in active sperms to swim up and travel from pellet to the surface layer of culture medium 16,17.

The data of sperm concentration of asthenozoospermia samples treated with Doum extract and NPs was similar to normozoospermia (control). This may ascribed for the influence of NPs on improving therapeutic effect of Doum extract on sperm motility, subsequently increased the number of motile concentrated sperm.

The data has showed decrease of motile sperm semen of asthenozoospermic patients. Sperm motility parameter plays a huge role in the evaluation of the fertility potential of a semen specimen. It is a critical parameter to assess the successful rate of fertilization 18.

The combination of medicinal herbs extract with ARTs has a significant influence to decreases the cost and successfully improve the outcome of infertility treatment. It has been noticed that different types of herbs have been investigated for their potential therapeutic influence on enhancing sperm quality for example; Phoenix dactylifera Pollen 19, Zingiber Officinale 11 and Glycyrrhiza glabra 15 and some other medicinal herbs.

Despite the fact that, the effectiveness of Doum extract has been exploited for some health aspects, few human research has been conducted on its effectiveness for male reproductive functions. This study has showed the positive effect of Doum extract with combination of silver NPs on sperm motility that resulted from its components. Doum fruit contains several components like essential oils, flavonoids, coumarins, saponins, and hydroxyl cinnamates 12,20. These active components have demonstrated potential therapeutic effect of healing activity in different health aspects 12,14.

The current study recorded enhancement in the percentage of MNS in men who complaining from asthenospermia, following in vitro activation. The in vitro activation technique and media used maybe contributed of this result 16.

Interestingly, the application of silver nanoparticles have boosted the efficiency of the beneficial effects of Doum extract activity on sperm motility. Recently, the implication of nanoparticles in biological activities has been growing increasingly all over the world 21.

The findings of this study proved that although Doum extract has improved the progressive motility of sperms but, the implication of silver NPs as a vehicles has significantly upgrade the influence of Doum extract on progressive motility of sperms. Thus, the application silver NPs may have bright future in ARTs to improve the outcomes of success rate of fertilization.

Conclusion: the study has showed for the first time that the implication of silver NPs has made significant difference on Doum extract effect on sperm progressive motility. Such finding may spot light on the beneficial effect of using silver NPs in ARTs to improve the outcome and increase the success rate of fertilization. The study sheds light on the potential influence of silver NPs as a potential new therapeutic approaches for implication in biomedicine; and fills the gap between traditional use of herbs and nanotechnology.

Ethical Clearance: none

Source of Funding : Self

Conflict of Interest : None

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Study of Isolated Bacteria from Burn Wound of Patients Attended Plastic Surgery and Burns Unit

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Abstract

Burns are one of the most public forms of trauma. Patients with severe thermal damage need instant focused maintenance in order to reduce morbidity and mortality. The aim of this study was to determine the prevalence of bacteria causing burn infection and their antibiotic resistance in Kirkuk city. This study carried out at the Plastic Surgery and Burns Unit in Azadi teaching hospital, Kirkuk-Iraq from January 2017- March, 2019. The study included 80 burned patient (50 females and 30 males), with age range 10-60 years. Swabs were taken from areas that seemed deep, with discharge, and the swabs were directly transported to the Microbiology Unit of the hospital. Data on age, gender and type of burn were also collected from the patients' clinical folder. The wound swabs were instantly cultured, then the isolated bacteria were subcultured for antibiotic sensitivity. The study showed that 82.5% (66 of 80) of patients enrolled in the study had positive culture of wound swab and 17.5% was negative. The highest rate of isolated bacteria from burn infection (38.75%) were from patients within the age group 29-29 year, followed by 23.75% in patient within the age group 10-19 year while the lowest rate (7.5%) was within the age group 50-60 year. The study presented that 62.5% (50 of 80) of patients enrolled were females and 37.5% (30 of 50) were males. In this study, bacterial culture was positive in 66 of 80 cases, with the predominant bacteria being *Staphylococcus aureus* (33.33%) (Table 3), followed by *Pseudomonas aeruginosa* (31.81%), *E. coli* (15.15 %), *Klebsiella* sp. (13.64%), *Proteus* sp. (3.03%) and (1.52%) for each of *Acinetobacter* sp. and *Enterobacter* sp. The study showed that most of *S. aureus* isolates were resistant to ampicillin, trimethoprim, ciprofloxacin, gentamicin and cefotaxime, most of *Pseudomonas aeruginosa* isolates were resistant to ampicillin, trimethoprim, ciprofloxacin and Augmentin, most of *E. coli* isolates were resistant to ampicillin, trimethoprim, Augmentin, trimethoprim, ciprofloxacin, ceftriaxone and tetracycline

Keyword: Burn; burn and wound infection; Plastic surgery.

Introduction

Burns are one of the most public forms of trauma (1). Patients with severe thermal damage need instant focused maintenance in order to reduce morbidity and mortality. Moderate to severe burn injuries requiring hospitalization account for approximately 100,000 of these cases, and about 5,000 patients die each year from burn-related complications(2). The survival rates for burn patients have improved substantially in the past few decades due to advances in modern medical care in specialized burn centers. Improved outcomes for severely burned patients have been attributed to medical advances in fluid resuscitation, nutritional support, pulmonary care, burn wound care, and infection control

practices(3). After initial period of shock, Infection of burn injuries is common complication, and is a major cause of death in burn patients. It is estimated that more than 70% deaths occurs in burn patients due to infection and these patients remain at risk of death until unless burn wound does not heal completely(4). Much progress has been made with respect to infection control and burn wound management, however, burn wound infection still poses a major clinical challenge in most developing countries, where wound site infections are a major source of post-operative illness and mortality among burn patients(5). The consequential effect of burn wounds contaminated with pathogenic bacteria can delay wound healing, cause wound breakdown and herniation of the

wound or complete wound dehiscence(6). Although in greatest cases the cause of infection is the patient's normal flora or exogenous contamination from contaminated wound dressing devices in or from the hospital setting, numerous groups of bacteria have been recorded to be related to wound infections. *Pseudomonas aeruginosa*, *Methylene Resistant Staphylococcus aureus* (MRSA), *Acinetobacter*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Citrobacter* sp., *Enterobacter* sp. and *Escherichia coli* were frequently linked with the wounds of burn patients(5-8). Although some studies have shown that adult females and children (1-9 years) are at a greater risk of burn-related injuries than adult males, burn wound is an important cause of disability and mortality in all ages and in both developed and developing countries(4,9). Very young children and the elderly have an increased risk of being burnt and have worse clinical outcomes than patients in other age groups(10). Individuals with deliberate self-inflicted burn injuries and the disabled have been shown to have more severe injuries and longer hospital stays than those with accidental injuries (11). So the aim of this study was to determine the prevalence of bacteria causing burn infection and their antibiotic resistance in Kirkuk city.

Material and Method

2.1. Patients:

This study carried out at the Plastic Surgery and Burns Unit in Azadi teaching hospital, Kirkuk-Iraq from January 2017 – March, 2019. The study included 80 burned patient (50 females and 30 males), with age range 10-60 years. Duration of patient admission ranged from 5 to 31 days. Wound swab was taken from each patient prior to wound bandage with hydrocortisone. Swabs were taken from areas that seemed deep, with discharge, and the swabs were directly transported to the Microbiology Unit of the hospita. Data on age, gender and type of burn were also collected from the patients' clinical folder. The wound swabs were instantly cultured on blood (Himedia) and MacConkey agar (Himedia), then incubated at 37°C for 24 hrs. After incubation, the colonial morphology of the colour, shape and general appearance of the individual colony on each of the plates was examined. 24 A representative single colony on the blood and MacConkey agar was gram stained and tested with indole and citrate, and Triple Sugar Ion test (TSI), urease and oxidase were performed to identify which bacteria species were present. Susceptibility testing

Temporarily, isolates were sub-cultured onto Mueller-Hinton agar plate using sterile swab sticks. The paper discs were gently but firmly placed on the inoculated plates using sterile forceps. The plates were incubated at 37oC for 24 hours after which zones of inhibition were measured and interpreted according to the Clinical and Laboratory Standard Institute.

Findings

The current study showed that 82.5% (66 of 80) of patients enrolled in the study had positive culture of wound swab and 17.5% was negative , Table 1

Table 1: Result of burn cultures

Culture result	No.	%
Positive	66	82.5
Negative	14	17.5
Total	80	100

The highest rate of isolated bacteria from burn infection (38.75%) were from patients within the age group 29-29 year, followed by 23.75% in patient within the age group 10-19 year while the lowest rate (7.5%) was within the age group 50-60 year. The study presented that 62.5% (50 of 80) of patients enrolled were females and 37.5% (30 of 50) were males, Table 2

Table 2: Distribution of studied patients according to age and sex

Age groups (years)	No.	Percentage
10-19	19	23.75
20-29	31	38.75
30- 39	16	20
40-49	8	10
50 -60	6	7.5
Male	30	37.5
Female	50	62.5

In this study, bacterial culture was positive in 66 of 80 cases, with the predominant bacteria being *Staphylococcus aureus* (33.33%) (Table 3), followed by *Pseudomonas aeruginosa* (31.81%), *E. coli* (15.15 %), *Klebsiella* sp. (13.64%), *Proteus* sp. (3.03%) and (1.52%) for each of *Acinetobacter* sp. and *Enterobacter* sp.

Table 3: Type of isolated bacteria from wound infection

Culture result	No.	%
Staphylococcus aureus	22	33.33
Pseudomonas aeruginosa	21	31.81
E. coli	10	15.15
Klebsiella	9	13.64
Proteus	2	3.03
Acinetobacter	1	1.52
Enterobacter	1	1.52
Total	66	100

The study showed that most of *S. aureus* isolates were resistant to ampicillin, trimethoprim, ciprofloxacin, gentamicin and cefotaxime, most of *Pseudomonas aeruginosa* isolates were resistant to ampicillin, trimethoprim, ciprofloxacin and Augmentin, most of *E. coli* isolates were resistant to ampicillin, trimethoprim, Augmentin, trimethoprim, ciprofloxacin, ceftriaxone and tetracycline, Table 4.

Table 4: Rate of antibiotic resistance against isolated bacteria

Culture result	No. of isolates	AMP	TMP	CIP	CRO	CTX	T	AK	AUG	CN
<i>S. aureus</i>	22	20	20	17	11	16	10	15	16	17
<i>P. aeruginosa</i>	21	21	18	17	15	16	20	18	19	14
<i>E. coli</i>	10	9	6	7	8	8	6	5	8	6
<i>Klebsiella</i>	9	8	8	6	5	6	5	4	7	4
<i>Proteus</i>	2	2	1	1	1	2	1	0	0	0
<i>Acinetobacter</i>	1	1	1	0	0	0	1	1	1	0
<i>Enterobacter</i>	1	0	0	0	1	1	1	0	0	1

Discussion

The current study showed that 82.5% (66 of 80) of patients enrolled in the study had positive culture of wound swab and 17.5% was negative, the highest rate of isolated bacteria from burn infection (38.75%) were from patients within the age group 29-29 year, followed by 23.75% in patient within the age group 10-19 year while the lowest rate (7.5%) was within the age group 50-60 year. the study presented that 62.5% (50 of 80) of patients enrolled were females and 37.5% (30 of 50) were males. This finding was in agreement with Forson et al (12) who found that 86% of burn swabs were positive with bacterial growth, 64% of these patients was females and the highest rate of infection (31%) were within the age group 21-30 year. Rao et al (13)

also recorded that the highest rate burn infection was in females (56.9%). The moderately higher number of burned patients in females may be due to their bigger contribution in kitchen doings. Chaudhary et al (13) also found that the age group below 30 years was most attended to hospital due to burns. This might be due to the point that the 21-30 group is the furthestmost active group, and most convoluted in outdoor activities. In this study, bacterial culture was positive in 66 of 80 cases, with the predominant bacteria being *Staphylococcus aureus* (33.33%) (Table 3), followed by *Pseudomonas aeruginosa* (31.81%), *E. coli* (15.15 %), *Klebsiella* sp. (13.64%), *Proteus* sp. (3.03%) and (1.52%) for each of *Acinetobacter* sp. and *Enterobacter* sp. This is agreement with Kehined et al (15) who found that *Klebsiella* spp. (34.4%) and *Pseudomonas* spp. (29.0%) were the most

common isolate from infected burn wounds and in agreement to other studies which report *Staphylococcus aureus* as predominant organism (16,17). Forson et al (12) found that *Pseudomonas* sp., *Staphylococcus aureus*, *Acinetobacter* spp, *Proteus mirabilis*, *Enterobacter* spp, *Klebsiella* sp., *Citrobacter* sp., *Klebsiella oxytoca* and *Proteus vulgaris* were the most common bacteria species isolated from burn wounds. Our finding was agreed with a study carried out by Patil et al (18) which also revealed *Pseudomonas* sp, *Staphylococcus aureus*, as the most common bacteria associated with burn wounds. The high prevalence of *Pseudomonas* sp. in this study may be due to the fact that the organism thrives well in a moist environment and *Staphylococcus aureus* is the predominant bacteria present originally on skin as well as in hospital environment (12). The antimicrobial susceptibility pattern of the different gram negative isolates from the burn patients revealed that *Pseudomonas* sp. was resistant to amikacin, ceftriaxone, ciprofloxacin and gentamicin. Findings from this study are similar to those of Shahzad et al (19) who reported varying resistance levels to amikacin (35%), ceftriaxone (85%), ciprofloxacin (70%) and gentamicin (97%). Alebachew et al (20) demonstrated that most of *Staphylococcus aureus* were resistant to penicillin G. All isolates were found to be multi drug resistant, and one isolate was resistant to all the tested drugs. Recent study noted that the resistance occur because of widespread and misuse of antibiotic, especially in the developing countries, the resistance profile of microorganisms has been altered significantly, also it could be attributed to several mechanisms, mostly related to antibiotics over use (21,22).

Conclusion

It was concluded that *S. aureus* and *P. aeruginosa* were the predominant isolated bacteria from burn wounds and most of them were resistant to available antibiotics.

Conflict of Interest: Non

Source of Findings: Self findings.

Ethical Clearance: This research was carried out with the patient's verbal and analytical approval before the sample was taken. According to this approval, all the samples were collected and the tests were carried out. A copy of the results of the tests was then given to the patients

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Non Significant Difference in *Parathyroid Hormone, Calcium or Phosphat* Levels Inpatients with Chronic Kidney Disease on *Conservative or Replacement Therapeutic Modalities*

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Abstract

Background: Chronic Kidney Disease is defined as abnormalities of kidney structure and function present for more than 3 months, mineral changes can occur early in chronic renal disease mainly phosphate, calcium hemostasis and secondary hyperparathyroidism

Objectives: Determine the levels of serum calcium, phosphate, alkaline phosphatase, parathyroid hormone and vit D3. In chronic renal failure and evaluate if there is any significant difference for those on HD and on conservative treatment

Methods: A cross sectional study was carried out among 150 uremic patients on conservative treatment or on haemodialysis. Take a full history from patients, full examination and proper investigation by using automated clinical chemistry analyser (Cobas integra 400 plus). Serum parathyroid hormone (PTH) and vit D3 by ELISA (Cobas e 400).

Results: A total number of 150 patients with CKD in medical department and Ibn Sina dialysis center at Baquba teaching hospital was investigated. The mean parathyroid hormone level was 144.87 pg/ml, An increase in PTH level above normal occurred in 41.34% of these patients, and 1.33% having hypoparathyroidism, and 57.33% of them are normal.

Hypocalcemia was found in 52.07% of patients, 1.33% have hypercalcemia and 46.6% was associated with normal serum calcium level. Age compared to those with normal serum calcium levels. majority of patients had high serum phosphate levels (54.6%). And normal phosphate level in 40% while the hypophosphatemia result 5.33% and mean phosphate level is 2.3 mmol/l.

Total serum protein mean is 61.51, a 37.4% of them had hypoproteinemia and 62.6% of them had normal serum protein. Also in our results show 97.33% of uremic patients have low vit D. **Conclusion:** the current study demonstrated that most uremic patients on conservative treatment and on haemodialysis having hyperparathyroidism, hypocalcemia, hyperphosphatemia, hypoalbuminemia and low level of vit D.

Keywords: Chronic renal failure-Secondary hyperparathyroidism.

Introduction

Chronic Kidney Disease is a common disease especially in patients with diabetes and hypertension [1]. so regular early renal function tests to detect an early stages of CKD is mandatory [2]. CKD is not only a risk factor for end stage renal disease, but may also increase cardiovascular morbidity and mortality [3]. The guidelines

of the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (K-DOQI) and the KDIGO guidelines recommend that all patients with eGFR below 60 ml/min/1.73 m² undergo evaluation for CKD-MBD by measuring PTH, calcium and phosphate [4].

All individuals with a glomerular filtration rate (GFR) <60 ml/min/1.73 m² for 3 months are classified

as having chronic kidney disease, irrespective of the presence or absence of kidney damage(1

GFR categories in CKD as follows

Stage	GFR level (mL/min/1.73 m ²)
Sta stage1	≥90
Stage2	60-89
Stage3	30-59
Stage4	15-29
Stage5	<15

In CKD the ability to excrete phosphate load by kidney is reduced that leading to hyperphosphatemia ,raised PTH, and reduction of 1,25(OH)2D with associated elevation in level of FGF-23.The ability to convert of 25(OH)D to 1,25(OH)2D is disturbed ,absorption of intestinal calcium reduced and elevated PTH. The kidneys are fail to work properly to PTH, which normally enhance phosphatemia and resorption of calcium ,or to FGF-23,which also stimulate phosphate excretion .Beside that, that is evidence of down regulation of vitamin D receptor at tissue level and of resistance to the action of PTH.

Haemodialysis replaces renal function , thereby improving renal bone disease . Different studies have demonstrated an improvement in secondary hyperparathyroidism (SHPT) after dialysis ;although parathyroid hormone (PTH) concentration remained elevated even in the presence excellent dialysis machines function .⁽⁵⁾ .Hypocalacemia and hyperphosphatemia are usually encountered in mineral bone disease ,Numerous studies have confirmed the reduced fracture risk after haemodialysis in patient who have CKD stage 5 and with renal transplant .⁽⁶⁾

There seems to be no doubt that pre dialysis mineral and bone disorder share and important role in the maintenance or development of HD alteration of bone remodeling, most HD patient suffer different types of pre existing bone disease that may continue after HD ⁽⁷⁾

Secondary hyperparathyroidism has been associated with metabolic bone disease which manifests as bone pain and skeletal fractures ^(8, 9,10)

material and methods

It is cross_sectional descriptive study done in medical department and Hemodialysis center of ibn_sena renal center at Baquba teaching hospital, Diyala/ Iraq. from julay 2017 to march 2018.

A total number 150 attending75 patient with chronic renal failure on conservative treatment(either preparing for dialysis or refused it and 75 patientson regular HD for 4 hours, three times aweek, irrespective to etiology.

102 were males and 48 females aged between 20 and 78 years were studied .

An investigator administered questionnaire was used to collect data from recruited patient. Data consisted of socio-demographic data including age, gender and living area. Information on smoking,alcohol ,treatment before, past medical history for possible etiology of kidney disease, time on dialysis, types of adjuvant treatment time since HD started and current medications were recorded.

About 5ml of blood was drawn from the cubital vein in sterile plain tube for creatinine, urea, calcium, phosphate, total serum protein, vit D, alkalin phosphatase, albumin and intact PTH .

The measurements of serum creatinine, albumin, calcium and phosphate were determined using automated clinical chemistry analyzer (cobas 400 plus) in the BTH, renal laboratory in IBN-sena center, while serum PTH assays were performed in BTH main laboratory using Enzyme linked immuno sorbent assay (ELISA) (cobas e 411).

Finding

The present study comprised 150 patients suffer from chronic renal failure attending to medical department and IBN_Sena haemodialysis center in Baquba teaching hospital during the period of study.

Age Distribution: As shownin Table1 the mean ages of our patients 52.2 yrs, the common age groups affected were 50_59 yrs of 54 patients, then age group 60_69 yrs were 34, then age group 40_49 were 24 and below age 39 were 32 and the least one is above 70es were 6 patients only.

Gender distribution : In our study, males made up the majority of the patients with 102 (68%) and females

48 (32%) as shown table 2

Residence distribution : in our study, there were 60patients (40%) from rural area and 90 (60%) from urban cities

Body mass index: the majority of patients in our study were over weight 88 (58.6%), and 22(14.6%) obese but only 40 (26.6%) normal

The Socioeconomic Status :As shown above in table 1, In present study, the patients were distributed as medium status in 96(64%), low status 44(29.33) and high status in 10(6.67%)

Table 1. socio_demographic data of study population

Variable	Count	Frequency%
Gender		
Male	102	68%
Female	48	32%
Age		
20_29	10	6.7%
30_39	22	14.7%
40_49	24	16%
50_59	54	36%
60_69	34	22.7%
70_79	6	4%
BMI		
Normal	40	26.66%
Overweight	88	58.67%
Obese	22	14.67%
Residence		
Rural	60	40%
urban	90	60%
Socioeconomic status		
Low	44	29.3%
Medium	96	64%
High	10	6.7%

Distribution according to duration of CKD and dialysis (in months):

Patients on conservative treatment 75 patients ,the duration of disease of 55 patients(73.3%) of them had renal impairment and uremia more than one year and 14 patients (18.7%)of than from 6m to 12 month and only 6 patients(8%) from 3 m to 6month

While patients on HD ,the duration of dialysis of 38 patients (50.7%) more than 24 months of HD,and 20

patients (26.7%) had 12- 20 months of HD and a only 17 patients (17.3%)less than 12 months of HD.

etiology of chronic renal failure (from the past medical history)

As shown in (table.2) which is documented in the file of the study subjects included hypertension in 122(81.33%) (m=88 and F =34),diabetes mellitus in 50 patients(33.3%) (m=36,F =14), renal stones in 40(%) (M=25,F=15), other geneto-urinary diseases eg UTI 50 (33.33%)(M=31 ,F =19) .bone diseases 5(M=4 ,F=1) history of fractures 4(M=3. F=1),

there are other causes of renal failure like obstructive uropathy present in 17 patients ,chronic glomerulo nephritis in 7 patients ,adult polycystic kidney disease in 4 patients and finally 24 patients of unknown causes.

Table 2:-Patients clinical characteristics

Duration of dialysis (months)		
1_3	1	1.3%
4_6	3	4%
7_12	13	17.3%
13_24	20	26.7%
>24	38	50.7%
Past medical history		
HT		
Yes	122	81.3%
No	28	18.7%
DM		
No	100	66.7%
Yes	50	33.3%
Genito urinaryinfection		
Yes	50	33.3%
No	100	66.7%
History of renal stone		
Yes	40	26.7%
No	110	73.3%
On conservative treatment		
More than 1 year	55	73.3%
6-1Y	14	18.7%
3-6 Month	6	8%

Drug administration :As shown in(table.3)a 50 patients (33.33%) have previous drug history of steroid administration and 120 patients (80%) having history of non steroidal drug uses and nearly 120 patient takes erythropoietin , all patients take calcium, alfa one and ferofolec medications ,Antihypertensive 122(82.4%,Oral hypoglycemic/insulin 50(33.3%) ,Calcium supplements 150 (100%)

Table.3 shown the drug administration

Drug history	Count	Frequency %
Steroid		
No	100	66.7%
Yes	50	33.3%
NSAID		
No	30	20%
Yes	120	80%
Drug treatment		
Ferro_folic acid		
No	0	0%
Yes	150	100%
Calcium		
No	150	100%
Yes	0	0%
Yes	150	100%
One alfa		
No	0	0%
Yes	150	100%
Erythropoietin		
No	30	20%
Yes	120	80%

biochemical characteristics of the study population:

Normal serum calcium levels were found in 70 (46.6%) patients while 78 (52%) patients had hypocalcemia and 2 (1.33%) cases have hypercalcaemia.

The median PTH was 145.62 pg/ml with majority of patients 86 patients(57.33%) having normal serum

levels. Hyperparathyroidism was found in 62 (41.33%) and hypoparathyroidism was seen in 2patients(1.33%) .the mean serum phosphorus level was 2.24 mmol/l with majority of patients having hyperphosphate ,82 paients (54.6%). Normal phosphate was seen in 60 patients(40%) and hypophosphatemia in another 8patients(5.4%).

Table.4: Biochemical characteristics of the study population

Variable	Count	Percentage	Mean (mmol	SD
PTN				
Hypo	2	1.33%		
Normal	86	57.33%	145.62	±255.16
Hyper	62	41.33%		
Mean corrected calcium			2.2mmol	±0.2

Cont... Table.4: biochemical characteristics of the study population

Calcium				
Hypo	78	52%		
Normal	70	46.6%		
Hyper	2	1.33%		
Mean of s. calcium in hypocalcemic patient			2.02	±0.1
Phosphate				
Hypo	8	5.4%	2.24mmol/l	±1.44
Normal	60	40%		
Hyper	82	54.6%		
Alk. Phosphatas				
Low	4	2.66%	123.23u/l	±86.51
Normal	100	66.66%		
High	46	30.66%		
Vit D				
Hypo	146	97.33%	38.03nmol/l	±12.14
Normal	4	2.33%		
Total serum protein				
Hypo	56	37.33%	61.51g/l	±8.57
Normal	94	62.66%		
s. albumin				
Hypo	112	74.66%	30.11g/l	±6.99
Normal	38	25.33%		

Discussion

The chronic kidney disease is a worldwide public health problem associated with high morbidity and mortality. As we know kidney transplantation from either a live or a cadaver donor is preferable treatment than conservative and dialysis therapy because transplantation provides a better quality of life and improved survival. However, even after otherwise successful kidney transplantation, mineral bone disorders do not always resolve⁽¹¹⁾.

Hyperparathyroidism In present study the hyperparathyroidism was found in (48.2%) patients, and most of them have acceptable value of PTH (52.8%) with mean (±SD) of 145.6(±255.16) at total values

while distributed as highest level with 192.5(±320.6) for conservative treatment and those with duration of dialysis of 4_6 months and 62.64(±38.38) the lowest for 7_12 months duration of dialysis with only 2(1.33%) has hypoparathyroidism. There is significant between the same group of duration of dialysis (p=.018). The mean of PTH for males were 149.5(±252.6) and for the females 137.3(±266.1) and the highest in age group below 39 yrs were 416 pg/ml and lowest in age group 70_79 yrs 10.05 pg/ml with significant correlation by anova table between age group (p value=.002). There is no significant between the means of age group 0 by the Duncan test.

The prevalence of HPT in a cross-sectional study done by El Maghraoui et al⁽¹²⁾ Which is higher than our study.

We found no correlation between HPT and duration, time on dialysis and eGFR which is similar to what was reported by mathumati et al. studies by Julian et al. appeared that, after 6 months HD patients exhibited a low bone mass, decreased mineral apposition rate, and delayed mineralization consistent with a pattern of a dynamic bone disease⁽¹³⁾. Cholahmossien⁽¹⁴⁾ reported hyperparathyroidism (PTH>60pg/ml) in 7.1% of the study population 3 months duration and was associated with a mean \bar{x} SD of 54 \bar{x} 18.68 months compared to a mean \bar{x} SD of 14.26 \bar{x} 8.34 months ($p<0.001$) in those with post HD PTH of ≤ 60 pg/ml. though our patients with elevated PTH had been on dialysis for a longer time (median duration 5 months) to HPT compared to those with normal PTH (median duration 12 months) or low PTH (median duration 10 months), the difference was not statistically significant.

The prevalence of hyperparathyroidism in our study was similar to that among CKD patients who were not yet on dialysis attending clinic at KNH done by Armando Torres, Aurelio PR, Maria TC et al, where 21.6% of the study population had HPT. These results suggest a persistence of hyperparathyroidism after starting regular HD programme. Pre HD hyperparathyroidism has been associated with persistent hyperparathyroidism after HD⁽¹⁵⁾.

Calcium level 78 patient (52%) of our study population had hypocalcemia with mean calcium levels of 2.02 mmol/l male were (38.7%) and females (13.3%), there are 70 patients (46.6%) have normal calcium level distributed as (29.3%) males and (17.3%) females with mean ca level of 2.22 and only 2 patients has hypercalcemia (1.33%), we found no significant correlation between age group nor duration of disease with hypocalcemia

A low pre hemodialysis PTH has been found to be a predictor of severe Hypocalcemia post HD although no significant correlation between the level of ca and PTH⁽¹⁶⁾.

This study agree with Iranian study done by taziki show most patient with low vit D and Ca⁽¹⁷⁾

phosphate level:-In our study there's no significant correlation was found between serum phosphate and different age groups or duration of disease and duration of dialysis. Sex, but significant with PTH ($p=.035$).

Kawarazaki et al., reported hypophosphatemia among 15% of HD 12 month duration. the mean PTH levels at 12 months duration of HD was higher 107(71.0-205.3)pg/ml compared to a mean of 2.24(± 1.44)(25.0-58.6)pg/ml at a median duration of (13-24) months HD in this study. The mean of serum phosphate in the early three months of dialysis was 2.300 mmol/l⁽¹⁸⁾

Serum phosphorus levels are usually elevated prior to renal dialysis and decreases rapidly following HD to within or below range for patients with normal kidney function in the first few post HD months.

VITD: The results in our study were 146(97.33%) have vit D deficiency and 4(2.33%) of patient were normal. This study results are goes with that done by Grahame j Elder(19) but not agree by study in japan by K Nakatsuka and Y Nishizawa⁽²⁰⁾.

ALP There is very significant ANOVA table correlation between ALP and Ca ($p=.000$), PTH($p=.000$) significant correlation with others.

Serum ALP is an independent predictor of bone density in all ranges of PTH⁽²¹⁾

Conclusion

the current study demonstrated that most uremic patients with uremia on conservative treatment and on haemodialysis having hyperparathyroidism, hypocalcemia, hyperphosphatemia, hypoalbuminemia and low level of vit D. and no significant difference in patients with Chronic kidney disease on conservative or replacement therapeutic modalities

Ethical Clearance: None

Source of Funding : Self

Conflict of Interest : None

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Correlated between Level of IL – 17 and Total Immunoglobulin (IgE) in Hay fever (H.F) Iraqi patients

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Abstract

Background : hay fever (Allergic rhinitis) are common chronic diseases worldwide., This study aimed at study the correlated between level of IL – 17 and total immunoglobulin (IgE) in Hay fever (H.F) Iraqi patients. **Patients and material** Allergic rhinitis was diagnosed by an experienced physician (NF) , The clinical history of the patient, any family history of the disease, a physical examination and the patient report (symptoms of rhinitis) were recorded, The total IgE levels and IL-17 in the serum samples were measured using Elisa kits , All analysis steps were performed according to the instructions of the manufacturer. **Results** Hay fever in females (53.5%) more than male (46.5 %) , also in control group ,the females more than male as percentage (56.3;43.7%) respectively, also current study founded more frequent of H.F within the second age groups (21-30)(39.6)% , whilst less prevalent in the youngest age (≤ 20) years as (23%) and (16.5,20.9) % frequent in the age groups 31-40 and ≥ 41 respectively, So the mean \pm S.D of IgE in sera of patients was more than control groups (121.38 ± 70.67 ; 32.37 ± 22.31) respectively. also level of interleukin-17 was highest in patients(401.28 ± 311.2) compare to control (362.41 ± 199.23)(P- Value H.S high significant). **Conclusion** Hay fever in females more than male ,Also current study conclude Hay fever more frequent within the second age groups (21-30) , whilst less prevalent in the youngest age (≤ 20) years ,So level of IgE in sera of patients was more than control groups. also level of interleukin-17 was highest in patients compare to control.

Keywords : *Interleukin – 17 ; total immunoglobulin (IgE);Hay fever (H.F);Iraqi patients*

Introduction

Hay fever (Allergic rhinitis) is a type of inflammation in the nose which occurs when the immune system overreacts to allergens in the air. is it characterized by sneezing, nasal congestion, nasal itching, and rhinorrhea, red, itchy, and watery eyes, and swelling around the eyes, These symptoms must be present for two or more days in a row and for more than an hour on most ⁽¹⁾.

Epidemiological data indicate that the prevalence of allergic rhinitis has increased in developing and developed countries over the last few decades⁽²⁾.

Allergic rhinitis is a diagnosis associated with a group of symptoms affecting the nose. These symptoms occur when you breathe in something you are allergic to, such as dust, animal dander, or pollen. Symptoms can also occur when eat a food that content allergic.

prevalence of H.F about 23.0–38.5% in 2012⁽³⁾,so it's very common in patients with asthma,13 with a reported prevalence of up to 100% in those with allergic asthma⁽⁴⁾

The first one is the most common form, and is associated with an immunoglobulin E (IgE)-mediated immune response against allergens⁽⁵⁾

Hay fever is triggered after allergen specific IgE and T helper cells recognize inhalant allergen in the environment. Some studies have shown that allergen exposure is protective against IgE mediated allergic disorders⁽⁶⁾

Differentiation of B cells into IgE-secreting plasma cells is a complex cascade of events in which cytokines play a crucial role⁽⁷⁾

IL-17, as a major family cytokine, is usually associated with autoimmune reaction or neutrophil inflammation. Nevertheless, it has been demonstrated

that allergic sensitization through the airway promotes a strong Th17 response and acute bronchial hyperactivity in mouse model of asthma⁽⁸⁾

Objective of this study : This study aimed at study the correlated between level of IL – 17 and total immunoglobulin (IgE) in Hay fever (H.F) Iraqi patients.

Patients and Material

Diagnosis of allergic rhinitis

Allergic rhinitis was diagnosed by an experienced physician (NF) , The clinical history of the patient, any family history of the disease, a physical examination and the patient report (symptoms of rhinitis) were recorded.

Blood sample collection: A 5 mL sample of peripheral blood was drawn from each participant by venous puncture and placed in tube , collected from Specialized Center of Allergy and Asthma, Baghdad , for IgE and IL-17 determination.

Determination of Total IgE and IL-17: The total IgE levels and IL-17 in the serum samples were measured using Elisa kits , All analysis steps were performed according to the instructions of the manufacturer.

Statistical analysis:

All statistical analysis were accomplished with SPSS system (virgin .19.0).

Finding

Table (1): Hay fever patients and control groups according to gender distribution.

Gender	Patients		Control	
	No.	%	No.	%
Male	39	46.5	14	43.7
Females	45	53.5	18	56.3
Total	84	100	32	100

In table (1) was founded Hay fever in females (53.5%) more than male (46.5 %), also in control group, the females more than male as percentage (56.3;43.7%) respectively .

Table (2): Hay fever patients and control groups according to gender distribution.

Age in years	Patients		Control	
	No.	%	No.	%
≤ 20	11	23	4	12.5
21 - 30	19	39.6	11	34.4
31-40	8	16.5	6	18.7
≥ 41	10	20.9	11	34.4
Total	48	100	32	100

Table (2) showed more frequent H.F within the second age groups (21-30)(39.6)% , whilst less prevalent in the youngest age (≤ 20) years as (23%) and (16.5,20.9) % frequent in the age groups 31-40 and ≥ 41 respectively.

Table (3): Level of total immunoglobulin I.g.E and Interleukin -17 in sera of Hay Fever patients .

Immunological test	Patients		Control		Significant
	Mean	S.D	Mean	S.D	
Total I.g.E	121.38	70.67	32.37	22.31	H.S
IL - 17	401.28	311.2	362.41	199.23	H.S
P- Value 0.01 , H.S high significant					

Results in (Table 3) showed the mean ± S.D of IgE in sera of patients was more than control groups (121.38 ± 70.67 ; 32.37 ± 22.31) respectively. also level of interleukin-17 was highest in patients(401.28 ± 311.2) compare to control (362.41 ± 199.23)(P- Value H.S high significant).

Discussion

Current study founded Hay fever in females more than male, This results agrees with Lasmar *etal.*, who reported a high prevalence of allergic rhinitis among Brazilian males in comparison with females⁽⁹⁾

. The results have been fully agreed with the data of ^(10,11) who showed allergic rhinitis is more common in men than in women. Additionally, ⁽¹⁰⁾ highlighted that the incidence of allergic rhinitis is even higher in men between the ages of 17 and 22 years.

Cazzoletti *etal.*, 2015 showed in his reported females were significantly less likely to report AR than males⁽¹²⁾ significant difference in age was observed between genders (males: 32.2±17.8 years; females: 41.4±18.9 years; $p=0.0027$). Additionally, the mean total IgE plasma levels were higher in males (413.0±143.0 IU/mL) than in females (147.9±98.0 IU/mL) ($p<0.0001$). These differences persisted even when males and females were stratified by age (up to or older than 20 years of age).

In Italy, results of ⁽¹³⁾ showed prevalence of H.F peaks around the age of 16–24 and decreases in the subsequent years up to the age of 65–70, this results was Fully compatible with results ⁽¹⁴⁾.

Results of ⁽¹⁵⁾ confirmed that females have a higher in the population aged between 20 and 44 years, but they also showed that the opposite is true when older age classes are considered.

Previous studies have found that the prevalence of H.F peaks around the age of 16–24 and decreases in the subsequent years up to the age of 65–70⁽¹³⁾

In Iran, results study of ⁽¹⁶⁾ showed prevalence of Hay fever in boys was higher than in girls ($P<0.05$). Results of ⁽¹⁷⁾ showed prevalence of H.F ranged from (10 -32) % in adults, whilst ⁽¹⁸⁾ showed children with aged 13-14 years had an overall 10% prevalence of H.F and those in Delhi had a prevalence of 11.6%.

Some studies using nasal allergen-provocation testing as the diagnostic standard have suggested that more than half of patients classified as having non allergic rhinitis on the basis of negative serum IgE or skin testing have “local allergic rhinitis” associated with production of allergen-specific IgE antibodies limited to the mucosa ⁽¹⁹⁾ Evaluating total IgE levels can be

useful to identify patients at risk of allergic rhinitis. ⁽²⁰⁾ showed significant elevation of serum IL-17 levels with an associated increase in serum IgE in the patients with H.F compared with controls. increased IL-17 serum levels might be considered a marker of allergy severity in patients with AR. The atopic state of the individual is the major determining factor that affects both the development and severity of AR as has been known for many years. One of the new function of IL-17 is its role in autoimmune disease.

Conclusion

Hay fever in females more than male, Also current study conclude Hay fever more frequent within the second age groups (21-30), whilst less prevalent in the youngest age (≤ 20) years, So level of IgE in sera of patients was more than control groups. also level of interleukin-17 was highest in patients compare to control.

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Influence of Disinfecting Solutions on the Surface Roughness of Zinc-Oxide Eugenol Impression Material

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Abstract

Background: dental impression can act as a role of transmission of infection. Difficulties in sterilizing impression by conventional methods have led to chemical disinfection as substitution. **Aim:** Evaluate the effect of natural disinfection solutions (Apple Vinegar and Lemon Juice) on surface roughness of Zinc – oxide eugenol impression material. **Materials and method:** fifty samples were prepared from zinc-oxide eugenol impression materials and divided into control group which exposed to saliva for five minutes and experimental groups which exposed to saliva then disinfectant agents (lemon juice and apple vinegar solution). Surface roughness of samples were analyzed for control group and experimental groups after 10 and 15 minutes. **Results:** data recorded from surface roughness test accounted lower mean values in control group and high mean values in lemon juice group followed by apple vinegar group. ANOVA test and LSD revealed that highly significant differences at $p < 0.001$ between tested groups. **Conclusion:** the study revealed that the immersion of ZOE in lemon juice solution and apple vinegar solution adversely affect surface roughness at 10 and 15 minutes

Keyword: ZOE impression, lemon juice, apple vinegar, surface roughness

Introduction

In dental clinics, dental impressions were contaminated with blood and saliva from oral cavity. Interaction between dental clinics and dental laboratories make impressions challenging items regarding cross infection. Guidelines in dental health care for infection control proposed that all prosthodontics items and prostheses must be rinsed, cleaned and disinfected before handling in the laboratory using an active disinfectant^{[1], [2]}.

Impression materials are widely used in the prosthodontics clinic. Zinc-oxide eugenol (ZOE) impression materials are widely used due to their ability to distribute pressure equally and accurately. ZOE impression provides good detailed reproduction without displacement of the soft tissues and is a mucostatic^[3].

Some of dental laboratories are sprayed the impression with surfactant solutions before pouring the model in order to rise the impressions' surface tension^[4].

Numerous methods for disinfection are proposed, disinfection of the impressions by immersing in chemicals take into consideration the most workable and dependable^[5]. There are many problems associated with chemical solutions that are harmful to health and environment as well as have unpleasant smell. Lemon juice is a natural disinfection solution and antiseptic. The juice can be directly used to the skin, and in home it is a bactericide and it is a beneficial component. Lemons, which are biologically active plant compounds that can aid in the healing of wounds, are rich in citrus flavonoids^[6]. Vinegar (acetic acid) could be substituted to conventional denture disinfectants, which is a solution presents low toxicity and low cost^[7]. It is effective when used as root canal chelators by removing smear layer. It is active in killing adherent microorganisms and does not cause mucosal damage^[8]. This study was designed to evaluate the effect of both lemon juice and apple vinegar disinfecting solutions on the surface roughness of ZOE impression material.

Materials and Method

Zinc oxide eugenol (England) impression materials were utilized in this study. The materials were manipulated according to the manufacturer's instructions. Plastic mold was made with dimensions of 1.5 cm length and 1.5 cm which was manufactured locally. ZOE impression materials were applied inside the mold; flat surfaces were obtained through pressing the mold against dried and cleaned glass slab. Samples were pressed using 1/2 kg weight to compress the excess materials out until samples were set. Figure (1)



Figure (1) final specimens for roughness test

The final set samples were divided as follows^[9]:

1-Control group: involved 10 samples exposed to diluted artificial saliva (50 % saliva in distilled water) for 5 minutes, then washed with distilled water and air dried.

2-Experimental groups: have 10 samples for each, exposed to diluted saliva for 5 minutes and then washed with distilled water and air dried. After that samples were immersed in commercially lemon juice solution and apple vinegar solution for ten minutes and fifteen minutes. Lastly the samples were washed under distilled water and dried. The surface roughness (Ra) was determined by surface roughness taster device (Pocket surf). The stylus was passed across the surface of the samples. Three readings were achieved from different areas of each sample. The average was calculated and Ra values were obtained in micrometers. Figure (2)



Figure (2) surface roughness device

Results

As shown in Figure (3) the minimum mean values were obtained in control group while the groups disinfected by lemon juice at 10 and 15 minutes showed high values followed by the group disinfected by apple vinegar at 10 and 15 minutes which accounted highest mean values.

Figure (3): Mean values of tested groups.

ANOVA test and least significant differences (LSD) accounted that highly significant differences at $P < 0.001$ between control group and experimental (lemon juice at 10 and 15 minutes) groups as in table (1, 2).

Table (1) ANOVA test between control and lemon juice groups

Groups	F	P-value	Sig
Between control with lemon juice 10 minute and group of lemon juice 15 minute	798.514	.000	HS

Comparison between lemon juice and control groups (LSD test) Table (2)

Groups		Mean Difference	Std. Error	Sig.
Control	Experimental group lemon juice 10 minute	-.20500-*	0.00802	.000
	experimental group lemon juice 15 minute	-.31600-*	0.00802	.000
Experimental group lemon juice 10 minute	experimental group lemon juice 15 minute	-.11100-*	0.00802	.000

Table (3,4) revealed that highly significant differences at $P < 0.001$ between control group and group of apple vinegar at 10 and 15 minutes by ANOVA test and LSD test.

Table (3) ANOVA test between apple vinegar groups and control group

Groups	F	P-value	Sig
Between control with experimental vinegar 10 minute and experimental vinegar 15 minute	815.509	.000	HS

Table (4) LSD test between control group and apple vinegar groups

Groups		Mean Difference	Std. Error	Sig.
Control	experimental vinegar 10 minute	-.32500-*	0.01066	.000
	experimental vinegar 15 minute	-.40700-*	0.01066	.000
experimental vinegar 10 minute	experimental vinegar 15 minute	-.08200-*	0.01066	.000

Discussion

Different methods have been utilized to create impression for complete denture involving selective pressure, functional, mucostatic etc. Final impression materials for complete edentulous arch include: (ZOE) paste, elastic impression and resilient (tissue conditioner material) ^[10]. A survey presented that (97 %) of Prosthodontics apply ZOE for taking final impression. It is a mucostatic impression and adheres easily to the soft tissues ^[11].

Disinfection of dental impression is a routine measure that be taken to prevent infection and contamination ^[12]. Disinfectant must be effective against microbial agent and no cause adverse effect on surface feature and dimensional accuracy of impression material ^[13]. The surface roughness of impression material was tested in this study as this characteristic influences surface free energy; the rough surface can cause a highly surface free energy that result in plaque formation. The importance of surface roughness in prosthetic substances are in contact with oral tissues; the most critical area of oral cavity and substances regarding to the long term clinical performance. Easily surface cleaning and less plaque formation were obtained in smooth surfaces of impression materials ^{[14], [15], [16], [17]}.

The results indicated that experimental groups (lemon juice and apple vinegar) recorded high Ra values of the surface roughness.

This could be attributed to the oily surface nature of the ZOE impression. The lemon juice and apple vinegar have chemically attacked the surface of the impressions and showed a noticeable damage which associated with the concentration of lemon juice and apple vinegar and time of exposure of impression to the lemon and vinegar ^[18].

Conclusion

The study concluded that the immersion of ZOE impressions materials in lemon juice solution and apple vinegar solution adversely affect surface roughness at 10 and 15 minutes

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Conflict of Interest: None

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Evaluate Some Biochemical Variables of Obese Iraqi Children in Baghdad City

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Abstract

Obesity in children is a medical problem that can affect their health in the future. These include serious cases such as type 2 diabetes, high cholesterol, high blood pressure - previously seen as adult diseases and many endocrine abnormalities including "thyroid dysfunction"

The study carried out at the pediatrics department, Baghdad hospital. Total (70) subjects selected from the coming patients and (30) healthy children. Age in each group between (5 month -10 years). The aim of this study assess the alteration in some sera levels of biochemical parameters (TSH, T3, T4, ALT, AST, ALP) with body mass index in obese child and compare the result with some parameters of non-obese children in Baghdad city. Thyroid function tests (TSH, T3, and T4) were measured in all subjects in both groups using enzyme linked fluorescent method to screening for thyroid dysfunctions. Liver enzymes test (ALT, AST, ALP) was determined by colorimetric method. Results show that the majority of obese children (42.8%) are aged in (6-10 years). This study statistically showed increased level of TSH among obese children compare with healthy control with a highly significant increase between them ($p < 0.0001$), while the levels of T3, T4 showed no influence on obese children ($p > 0.05$). There was no significant increase in serum (AST, ALT, ALP) in this study in obesity children and healthy children ($p > 0.05$). Increased weight in children may be caused by the main imbalance in the thyroid hormones especially thyroid stimulating hormone (TSH). "Hypothyroidism" in obese children when left untreated may lead to uncontrollable diseases such as mental retardation. Thyroid hormones should be examined in obese children regularly to check for any effects that lead to diseases that are difficult to control.

Keywords: *Body mass index (BMI), thyroxine (T4), thyroid-stimulating hormone (TSH), triiodothyronine (T3), aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (Alp)*

Introduction

One of the most emerged serious public health concerns in the 21st century is overweight and obesity among children, which is a morbidity and mortality predictor of adulthood obesity^[1]

Childhood obesity is a worldwide health problem. Obesity has turned into a worldwide plague in both developed and developing countries throughout the most recent couple of decades from 1980, and its prevalence has increased from (4.2%) in 1990 to (6.7%) in 2010 and is expected to reach 9.1% or 60 million by 2024^[2].

Obesity is an excessive increase in weight with increase in body fat. Causing the patient to be exposed to several diseases may be dangerous and chronic,

including heart disease and type II diabetes, as well as various cancers. Obesity is measured in many ways, the most important of which is known as the body mass index (BMI - body mass index), a tool to calculate the weight of body length.^[3]

Weight a problem is related to many endocrine abnormalities which includes thyroid dysfunction. The thyroid gland is important for promotes human fetal improvement and supply of the hormones triiodothyronine (T3) and thyroxine (T4). The thyroid usually secretes T4, which is converted to the extra active T3 by using in tissue deiodinase enzymes. Thyroid hormones control metabolic processes, cardiac reproduction and regular fetal mind improvement^[4]. In recent years, there has been an growing attention to

thyroid characteristic in pediatrics overweight sufferers [5]. Thyroid function test form an important influence on study of the relationship between thyroid disorder and weight control.

Weight gain and thyroid hormones appear like closely related. There was several studies found that disorder in thyroid-stimulating hormone (TSH) levels increase weight gain, other study shows no-relationship between them [6]. Thyroid hormones alter basal metabolism, thermogenesis and play and crucial role in lipid and glucose metabolism, meals consumption and fat oxidation also thyroid disorder is associated with body temperature adjustments, body weight and composition. [7].

Patients and Method

The case carried out at the pediatrics department, Baghdad hospital. Total (70) sample were selected from the coming patients and (30) healthy children there age between (5 month -10 years). BMI was calculated as weight divided by squared height (kg/m^2). For pediatric obesity degree was evaluated using the BMI percentiles depend on age and sex using the definition of the (International Task Force for Obesity in Childhood) [8] [9]. They study were divided into two groups. Both groups were matched according to their age and sex. Children with a body mass index (BMI) maximum than or equal to 85th centile and 95th centile were situation in the obese class, while other children with BMI greater than or equal to 5th centile but less than 85th centile standards were assorted as the normal weight groups and describe as control groups. Children with diabetes

mellitus and genetic endocrine causes of obesity were excluded in this study. Demographic data was collected regarding age and gender. Thyroid function tests (TSH, T4, and T3) were measured in all subjects in both groups using an enzyme linked fluorescent assay method as screening for thyroid dysfunctions. Reference values for thyroid function test were as follows: TSH (IU/ml), reference range 0.5–5 and T3 (nmol/l , reference range 1.3–2.75), T4 (nmol/l reference range 57.9–167). Liver enzymes including aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (Alp) were measured by colorimetric method.

Results and Discussion

Figure (1): Age distribution of obese children in research

1*(5 - 11 month) , 2*(1- 5 years) , 3*(6 - 10 years)

The study sample included (70) obese children with mean age 4.79 ± 0.2 years include 25.8% was (5 -11 month), 31.4% (1-5 y) and 42.8% there are between (6 -10y) respectively.

The results showed in Figure 1 that the majority of obese children (42.8%) were between the ages of (6-10) years. Obesity is a complex disorder that involves increasing the amount of body fat, which spreads like a global epidemic due to financial conditions and fast food also genetic predisposition has a significant role in children's ability to gain weight. Numerous studies started to concentrate on obesity disease as well as nutrition. Extremely obese leads health problems related to weight gain [10].

Table (1) : BMI Distribution of the study children

Variable	Obese(n = 70)	Normal Weight (n = 30)	p – value
Weight(Kg)			
(5-11m)	10.67± 0.78	4.6±1.2	0.001
(1-5y)	21.4± 2.7	10.4± 3.2	
(6-10y)	35± 5.6	17± 4.9	
Height(cm)			
(5-11m)	60.75± 8.8	50.33±33.9	0.001
(1-5y)	96.4±20.2	78.56± 44	
(6-10y)	117 ±45.9	102.45±97	

Cont... Table (1) : BMI Distribution of the study children

BMI (Kg/cm ²)			0.001
(5-11m)	27.8	16	
(5-11m)	22.8	16.4	
(1-5y)	27.9	16.3	

BMI: Body Mass Index

Table (2) illustrated those children who BMI between **(16-16.3)** kg/cm². That puts them in the **(53rd percentile)** which indicates that they are **at a healthy weight**. BMI between **(27.8-27.9)**. That puts them in the **(99th percentile)** which indicates that they are **obese children**. Obesity is a serious medical condition affecting children and adolescents. Obese children are above normal weight for their age and height. BMI is especially useful in identifying children at high risk for weight gain as they age. An increase child's BMI in percentile rank over one year is a possible sign that child is at risk of becoming overweight.^[11]

Table (2) : Laboratory findings and demographic between groups.

variables	Obese n=(70)	Normal weight n=(30)	p-value
boys	30(42.8%)	13(43.3%)	0.43
girls	40(57.1%)	17(56.6%)	0.33
T3(nmol/l)	2.37± 0.3	1.87± 0.1	0.34
T4(nmol/l)	74.6±24.7	66.3± 12	0.44
TSH (IU/ml)	6.97± 2.2	3.1±1.5	0.00
S.ALT(U/L)	23±2.2	25±3.1	0.23
S.AST(U/L)	21± 1.4	27± 4.8	0.28
S.ALP(U/L)	260±70.98	183±60.3	0.30

Table (2) demonstrated that TSH levels were significantly higher in obese children compared to healthy children ($p < 0.001$) while T3, T4 remains in the normal range among both groups.

Liver function test include (ALT), (AST) and (ALP) was done in both obese children and healthy children. There was no significant differences regarding ALT, AST, ALP between obese child and those with control subject, in spite elevation in enzyme (ALP) levels in obese children compared to healthy weight groups.

The present studies explain that alterations in thyroid function can frequently be observed in children being overweight or obese. This result is in agreement with other study.^[12]

In hypothyroidism change in thyroid function may result with normal regimen regulation (T4 at a minimum normal rate and increased TSH hormone though within normal range) in increasing changes in energy consumption with continuous increases in body mass index and weight. An increase in fat mass and TSH values may increase serum(leptin) level^[13]

In the adipose tissue(leptin) which works physiologically, regulates the energy balance by informing the central nervous system. It modifies neuro-endocrine and behavioral responses to over-nutrition, thus regulating food consumption and energy expenditure. Some of the hypotheses also indicate a high conversion from T4 to T3 in patients with central obesity due to increased diiodase activity as a compensatory fat-breaking mechanism to improve energy expenditure. [14]

Many studies have shown that children who are overweight or obese are more likely to develop liver disease at later times than their normal weight counterparts. The present study shows poor correlation of liver enzyme test(ALT,AST,ALP) in development of obesity. Obesity is an important risk factor for many conditions including (non-alcoholic fatty liver disease). The incidence of fatty liver disease in children is increasing day by day, with about 30% of the world's population now suffering from this disease and the incidence of fatty liver disease non-alcoholic about 9.6% on children. Non-alcoholic fatty liver disease is one of the most common liver diseases and can lead to serious health problems that may reach cirrhosis in children and end up with liver cancer or liver failure in the elderly. [15]

In most patients, non-alcoholic fatty liver disease does not cause any symptoms. [16]Non-alcoholic fatty liver is one of the diseases that affects children and adolescents, especially those who are obese, and high levels of cholesterol, as a result of eating foods high in saturated fats, especially fast food and sugar-rich foods

The association between liver enzymes and BMI may be affected with the presence of other enhancing elements, together with weight gain, diet, duration of remedy and compliance with medication intake on liver integrity in future if the obese child left without medical follow-up. The accumulation of fat in the liver leads to fibrosis, which can eventually lead to the liver completely from functioning. The risk is that symptoms may not appear until the damage is done. Doctors do not know exactly what causes fatty liver disease, but people are more likely to have liver problems when they are overweight, especially in childhood.

Conclusion

Early detection of hypothyroidism and early treatment is permitted to the extent possible to prevent

irreparable damage to the nervous system in the growth stage also choosing healthy foods for infants and young children is crucial because the preference for certain foods is established early in life. Feeding with energy-rich, high-fat, and high-sugar and high-salt foods is a major contributing factor to childhood obesity

There is great importance from an early age to maintaining a balanced diet and a proper weight and there is great importance for the early diagnosis of childhood fatty liver in order to avoid potential complications in adulthood

Conflict of Interest: None

Source of Findings: Self

Ethical Clearance: Nil

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Implication of Silver Nanoparticles to Enhance the Potential Therapeutic Activity of Doum Palm (*Hyphaene Thebaica*) Extract for *in vitro* Sperm Activation of Asthenozoospermic Infertile Men

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Abstract

Male infertility is a worldwide public health issue which affected a significant proportion of humanity. Many herbs extracts have been well documented as a therapy for infertility. This study has uniquely examined for the first time, the role of the bioactive effect of Doum Palm extract using silver nanoparticles (NPs) on improving the sperm motility of infertile human male. Semen samples of ten patients diagnosed with asthenozoospermia were collected. Semen samples were collected from ten voluntary normozoospermic males used as positive control. Doum Palm extract with and/or without silver NPs was investigated using *In vitro* sperm activation layering technique. The present study has demonstrated that there was a significant ($p < 0.05$) effect of Doum Palm extract with and without using silver NPs on active sperm motility when compared with control semen samples. Interestingly, this study has found that the application of silver NPs as a drug delivery vehicles for Doum Palm extract has a significant implication on enhancing the sperm motility *in vitro*. This finding suggested that the implication of NPs technology in assisted reproductive techniques (ARTs) may have a great future on improving sperm quality.

Key words: Male infertility, Doum Palm (*Hyphaene thebaica*), Extract, Silver nanoparticles.

Introduction

Male infertility is a global growing problem among men, may occur due to the influences of several factors including; abnormal sperm function, production of decreased sperm count, illnesses, injuries, chronic health issues and choices of lifestyle 1. In general, the quantity and quality of sperms have impact effect on man fertility. For example it is difficult for a man to cause pregnancy if his ejaculated sperm number was low or if sperm reveal poor morphological and functional quality 2.

Since the breakthrough of ARTs in 1970s, and the understanding of infertility term has dramatically

changed. In particularly, the highly remarkable role of intensify the opportunities for infertile couples to have babies can be achieved by using the intracytoplasmic sperm insemination (ICSI) and the *in vitro* fertilization technique (IVF) 3,4. Despite the advantages of ARTs, the success of ARTs are not guaranteed, and patients often have to undergo more than one cycle of harmful medication treatment before they are successful. Therefore, the application of medicinal herbs as an alternative of medicinal in ARTs could successfully increase the outcome of infertility treatment and decrease the cost 5,6,7. There are many medicinal herbs extract have been remarkably investigated for their therapeutic activity on improving sperm quality for ARTs. In fact medicinal herbs have been widely examined to boost different aspects of health care due to their bioactive contents, as they possess different biological therapeutic activities including; antimicrobial, antioxidant and anticancer effect 8,9,10,11. One of these herbs is Doum

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Palm (Hyphaene Thebaica) extract. Doum Palm is natively found in the tropical countries, particularly Upper Egypt. Doum Palm also known as gingerbread tree its edible oval fruit [12]. Furthermore, the bioactive effect of Doum extract has been investigated in ARTs for successfully improving the outcome of infertility treatment.

Several studies have suggested that Doum extract has potential effect on improving male reproductive function [3,13]. Clearly, these studies have adapted the traditional and common investigation method of combination of medicinal herbs extract with ARTs. Whereas, in current study the main objective was to boost the outcome of success rate of in vitro infertility treatment via the combination of nanotechnology with ARTs. The combination of medicinal herbs extract with ARTs using nanoparticles technology is novel and has not been well studied. Therefore, the study will implicate the silver NPs as a delivery vehicles for Doum extract for in vitro treatment of asthenozoospermic infertile men.

Materials and Method

Preparation dried extract of Doum Palm

Doum Palm fruit has been obtained from the local market. The Doum fruit was dried and grinded. 100 ml of distilled water was added to 20 gm of Doum Palm powder and refluxed for 3 hours. Once the mixture was homogenized and the powder was completely dissolved, the mixture was filtrated by using a sheet of gauze in a clean suitable flask. Finally, the filtrated solution transferred to sterile Petri dishes (Falcon, USA) and dried on an incubator at 37 °C [14].

Preparation of aqueous Doum Palm extract for in vitro sperm activation

The aqueous working solution of Doum extract was prepared by dissolving 0.5 mg of dried Doum extract with 10ml of phosphate buffer solution (PBS) (Sigma-Aldrich, USA) in plastic test tube (Falcon, USA) contained broad spectrum antibiotic. For the purpose of in vitro sperm activation, a 20% of Doum aqueous working solution has prepared by adding 8 ml of PBS to 2 ml of the aqueous working solution of Doum extract. The aqueous solution was filtered using Millipore (Millipore, USA) 0.45 µM. Media was adjusted at pH 7.5-7.8 and stored at 25 °C.

Mixture preparation of silver nitrate nanoparticles with aqueous Doum extract for in vitro sperm activation

The mixture of silver nitrate NPs with aqueous Doum extract was prepared by adding 0.58 mg of silver nitrate to 50 ml distilled water. Then, the working solution of silver nitrate nanoparticles with aqueous Doum extract for in vitro sperm activation 10 ml of aqueous Doum extract was mixed with 50 ml of the solution of silver nitrate. Later on, the final mixture of aqueous Doum extract and silver nitrate NPs kept on magnetic stirrer until the brown colour of mixture changed to the brown red colour.

Experimental design

Following the ethical approval and informed consent from all donors, semen samples were collected from ten patients diagnosed with asthenozoospermia enrolled in the Biotechnology Research Center and private Laboratory through the period from December 2018 till March 2019. Each semen sample was divided into three groups. The semen of the first men group (i.e. control group) were treated with PBS only. Semen of the second group were treated with Doum extract only (i.e. Extr. group) and semen of the third group were treated with a solution containing Doum extract and silver NPs (i.e. Extr. +NPs group). Samples were incubated for 1 hour at 37 °C in CO2 incubator. The inverted microscope was used to examine the Sperm concentration, sperm motility and morphologically (Wild Herrbrug, Switzerland) with 40X magnification. The examination was performed in each group before and following in vitro incubation using layer technique as described by Al-Dujaily et al., [15].

Statistical Analysis

GraphPad Prism 8.0.2 software was used for data analysis. The relationships between parameters and differences between groups were tested for significance using Ordinary one-way ANOVA of One-way ANOVA with Sidak's multiple comparisons test. Data have been shown as Means ± standard error of the mean (M ± SEM). p values of <0.05 were considered significant.

Results

The results revealed a significant improvement on concentration of sperm, number of motile sperm of grad A in both treatment groups (Doum extract only group or Doum extract with NPs group) and in morphologically

normal sperm (%) when compared with non-treated group i.e. control group (Table -1)

The sperm concentration after in vitro activation was significantly ($P < 0.05$) decreased in control group compared to pre-activation and post- activation groups. The same observation was reported when using Doum extract medium alone. However, there was no significant differences in sperm concentration between pre-activation (60.45 ± 9.80) and post-activation when the medium containing both the Doum extract and NPs (56.0 ± 11.61).

There data showed highly significant ($p < 0.0001$) increase in the number of motile sperm of grad (A) of sperm activated in vitro in Doum extract with NPs medium compared with group treated with Doum extract only (Table-1 and Figure -1A). In addition, there was a significant increase in the number of motile sperm of grad B in both treatment groups (Doum extract only group or Doum extract with NPs) when compared with non-treated group i.e. control group; $p = 0.0025$, $p < 0.0001$, respectively (Figure B). Likewise, the data has showed a significant increase in the number of motile sperm of grad (B) in Doum extract with NPs group

compared with group treated with Doum extract only $p = 0.0029$ (Figure B).

No significant differences on grad C of sperm motility in both treatment groups (Doum extract only group or Doum extract with NPs) when compared with non-treated group, $p = 0.6845$, $p = 0.4631$ respectively, (Figure C). Although there was slightly differences in the number of motile sperm of grad (C) in Doum extract with NPs group compared with group treated with Doum extract only but it was not insignificant ($p = 0.074$, Figure C).

On the other hand, the result revealed a significant elevation in the number of motile sperm of grad D in both treatment groups (Doum extract only group or Doum extract with NPs) when compared with non-treated group i.e. control group $p < 0.0001$ for both groups (Figure D).

The data of present work revealed that there was a significant ($p < 0.05$) elevation in MNS percentage post-activation compared to pre-activation. However, there was no significant ($p > 0.05$) different in the percentage of MNS post-activation using any medium as shown in table -1.

Table-1: Effect of Doum Palm extract with and without NPs on certain sperm parameters using layer technique for in vitro activation

Certain sperm parameters		Pre activation	Post activation		
			Control	T1	T2
Sperm concentration (m/ml)		$60.45 \pm 9.80a$	$40.2 \pm 10.68c$	$51.6 \pm 10.66b$	$56.0 \pm 11.61a$
Sperm Motility (%)	Active sperm motility A	$2 \pm 1.67d$	$8.1 \pm 1.85c$	$15.5 \pm 1.72b$	$26.0 \pm 6.58a$
	Active sperm motility B	$10.3 \pm 7.52d$	$15.7 \pm 2.71c$	$23.3 \pm 3.92b$	$30.8 \pm 6.23a$
	Inactive sperm motility C	$35.7 \pm 8.43b$	$27.6 \pm 2.22a$	$29.7 \pm 3.74a$	$24.8 \pm 6.73a$
	Immotile sperm D	$52 \pm 6.72d$	$48.6 \pm 4.87c$	$31.5 \pm 5.04b$	$19.4 \pm 9.13a$
Morphologically Normal sperm (%)		$40.56 \pm 8.74b$	$54.4 \pm 7.48a$	$55.7 \pm 9.8a$	$56.4 \pm 8.66a$

Values are expression as mean \pm SEM

Number of Samples = 20

T1=medium with Doum extract only. T2= medium with Doum extract and NPs

Figure 1: Silver NPs enhanced the potential therapeutic effect of Doum extract on progressive motility of sperm of asthenozoospermic male. A) Data showed both extract alone group and extr+NPs group has significant effect on sperm motility of grad (A) when compared with control $**p = 0.0011$, $****p < 0.0001$ respectively. B) Data showed both extract alone group and extr+NPs group has significant effect on sperm motility of grad (B) when compared with control $**p = 0.0025$, $****p < 0.0001$ respectively. C) Data showed both extract alone group and extr+NPs group has NO significant effect on sperm motility of grad (C) when compared with control. D) Data showed both extract alone group and extr+NPs group has significant lower effect on sperm motility of grad (D) when compared with control $****p < 0.0001$ for both groups.

Discussion

Following in vitro activation, there was a significant decrease in sperm concentration in both control samples and medium containing Doum extract only. This is due to the failure of the dead and in active sperms to swim up and travel from pellet to the surface layer of culture medium 16,17.

The data of sperm concentration of asthenozoospermia samples treated with Doum extract and NPs was similar to normozoospermia (control). This may ascribed for the influence of NPs on improving therapeutic effect of Doum extract on sperm motility, subsequently increased the number of motile concentrated sperm.

The data has showed decrease of motile sperm semen of asthenozoospermic patients. Sperm motility parameter plays a huge role in the evaluation of the fertility potential of a semen specimen. It is a critical parameter to assess the successful rate of fertilization 18.

The combination of medicinal herbs extract with ARTs has a significant influence to decreases the cost and successfully improve the outcome of infertility treatment. It has been noticed that different types of herbs have been investigated for their potential therapeutic influence on enhancing sperm quality for example; Phoenix dactylifera Pollen 19, Zingiber

Officinale 11 and Glycyrrhiza glabra 15 and some other medicinal herbs.

Despite the fact that, the effectiveness of Doum extract has been exploited for some health aspects, few human research has been conducted on its effectiveness for male reproductive functions. This study has showed the positive effect of Doum extract with combination of silver NPs on sperm motility that resulted from its components. Doum fruit contains several components like essential oils, flavonoids, coumarins, saponins, and hydroxyl cinnamates 12,20. These active components have demonstrated potential therapeutic effect of healing activity in different health aspects 12,14.

The current study recorded enhancement in the percentage of MNS in men who complaining from asthenospermia, following in vitro activation. The in vitro activation technique and media used maybe contributed of this result 16.

Interestingly, the application of silver nanoparticles have boosted the efficiency of the beneficial effects of Doum extract activity on sperm motility. Recently, the implication of nanoparticles in biological activities has been growing increasingly all over the world 21.

The findings of this study proved that although Doum extract has improved the progressive motility of sperms but, the implication of silver NPs as a vehicles has significantly upgrade the influence of Doum extract on progressive motility of sperms. Thus, the application silver NPs may have bright future in ARTs to improve the outcomes of success rate of fertilization.

Conclusion: the study has showed for the first time that the implication of silver NPs has made significant difference on Doum extract effect on sperm progressive motility. Such finding may spot light on the beneficial effect of using silver NPs in ARTs to improve the outcome and increase the success rate of fertilization. The study sheds light on the potential influence of silver NPs as a potential new therapeutic approaches for implication in biomedicine; and fills the gap between traditional use of herbs and nanotechnology.

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Conflict of interest : None

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Assessment of melatonin and oxidant-antioxidant markers in infertile men in Thi-Qar Province

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Abstract

The present study aimed to investigate the level of melatonin MDA, GSH in infertile men compare with the fertile men in Al-Hussein hospital in Thi-Qar province ,Iraq, from 10th of January 2019 to 10th of March 2019. The study included 65 infertile men whose age were between 21-40 years , also included 35 healthy individuals with same age who visited blood bank in Thi-Qar province for blood donation. Five ml of blood sample was collected subject enrolled from each one for measurement of serum albumin, reduced glutathione (GSH), malondialdehyde (MDA) by spectrophotometric kit. as well as estimation of melatonin by ELISA technique. The study showed that melatonin was reduced significantly in infertile men (226.5 ± 11.9 pg/ml) as compared with healthy control (381.6 ± 12.8 pg/ml). Also there was significant increase in the level of MDA in infertile men (4.15 ± 2.34 pg/ml) as compared with healthy control (1.71 ± 0.44 pg/ml). The study showed that the lowest mean level of glutathione was recorded in infertile mean (31.8 ± 2.5 mmol/l) as compared with healthy control (63.7 ± 2.1 mmol/l) with a significant relation ($p < 0.05$). The study showed that the minimum level of albumin was recorded in infertile mean (4.24 ± 0.29 g/dl) as compared with healthy control with highly significant relation.

Key words: Melatonin; MDA; Glutathione; Male infertility.

Introduction

Infertility has been defined as failure to conceive after frequent unprotected sexual intercourse for one or two years. Male factor infertility accounting for 50% of cases⁽¹⁾. It is very important to identify the factors/conditions which affect normal sperm functions. Infertility is the problem of 15% of young couples in different societies⁽²⁾. Therefore, any assistance in this matter would promote the health status of families and society⁽³⁾. Since spermatogenesis and oogenesis are the basis of fertility, increasing our knowledge about factors that are involved in them, would be helpful in solving the fertility problem⁽⁴⁾. It is very important to identify the factors/conditions which affect normal sperm functions.

Melatonin (N-acetyl-5-methoxy-tryptamine), Melatonin (N-acetyl-5-methoxytryptamine), a tryptophan derivative secreted by the pineal gland, is a highly evolutionarily conserved molecule – present virtually in all organisms. It is produced predominantly

during the dark phase of the circadian cycle. This hormone plays an essential role in the regulation of circadian changes in various physiological aspects and neuroendocrine functions. In mammals, melatonin can affect the process of sexual maturation and reproductive functions by stimulating the HPG axis⁽⁵⁻⁷⁾.

Oxidative stress (OS) is one of the known factors which is involved in reproduction. The imbalance in the production of oxidants or free radicals and antioxidant molecules in the body would result in OS state⁽⁸⁾. Oxidative stress which is formed by the breakdown of the balance between free radicals and antioxidants in favor of free radicals, plays a significant role in the pathogenesis of many diseases and mechanisms of complications⁽⁹⁾. The association between decreased sperm quality and increased ROS level in infertile men has previously been shown. On the other hand, it is reported that reduction of antioxidant level results in infertility and antioxidants could be used for treatment of infertility in men⁽¹⁰⁾.

Materials and Method

Subjects: The presented study included 65 infertile men whose age were between 21-40 years who attended to the Al-Hussein hospital in Thi-Qar province ,Iraq, from 10th of January 2019 to 10th of March 2019 with the same age groups who visited blood bank in Thi-Qar province as control group.

Method

Five ml of blood sample was taken by vein puncture from each subject enrolled in this study. Blood samples were placed into sterile test tubes, after blood clotting, centrifuged at 3000 rpm for 15 minute then clot removed and remain re-centrifuged at 3000 for 10 minute and the obtained serum were aspirated using mechanical micropipette and transferred into clean test tubes which labelled and stored in deep freeze at -20 °C until analysis. serum melatonin was analysed by enzyme linked fluorescent assay (ELFA) technique. serum albumin, reduced glutathione, MDA levels were measured by spectrophotometric kit.

Statistical analysis: Statistical analysis was performed using SPSS-21 Unpaired t test was done to evaluate significant variance between means. $P < 0.05$ was considered statistically significant.

Finding

The study showed that a significant decrease of serum melatonin ($p < 0.05$) in infertile men (226.5 ± 11.9 pg/ml) as compared with healthy control (381.6 ± 12.8 pg/ml).

Table 1: Level of melatonin in infertile patients and healthy individuals

Melatonin level (pg/ml)	Infertile patients	Control group
No.	65	35
Mean \pm SD.	226.5 ± 11.9	381.6 ± 12.8
T. Test: 6.65 P. value: 0.0001 Highly significant		

* P. value < 0.01 : highly significant

The study presented that the highest mean level of MDA was found significantly in infertile men (4.15 ± 2.34

pg/ml) as compared with healthy control (1.71 ± 0.44 pg/ml), P. value: 0.0015, Figure 1.

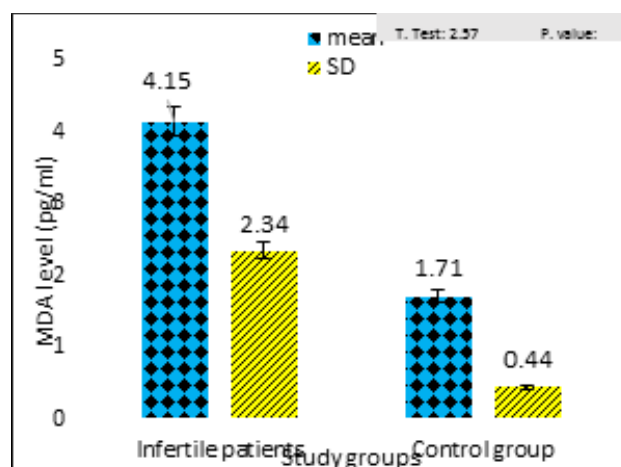


Figure 1: Level of MDA in infertile patients and healthy individuals.

The study showed that the maximum level of albumin was recorded in infertile mean (4.24 ± 0.29 g/dl) as compared with healthy control with highly significant relation ($p: 0.009$), Table 2.

Table 2: Level of albumin in infertile patients and healthy individuals

Albumin level (g/dl)	Infertile patients	Control group
No.	65	35
Mean \pm SD.	4.24 ± 0.29	5.22 ± 0.22
T. Test: 2.55 P. value: 0.009 highly significant		

The study showed that the lowest mean level of glutathione was recorded in infertile mean (31.8 ± 2.5 mmol/l) as compared with healthy control (63.7 ± 2.1 mmol/l) with a significant relation ($p < 0.05$), Figure 2.

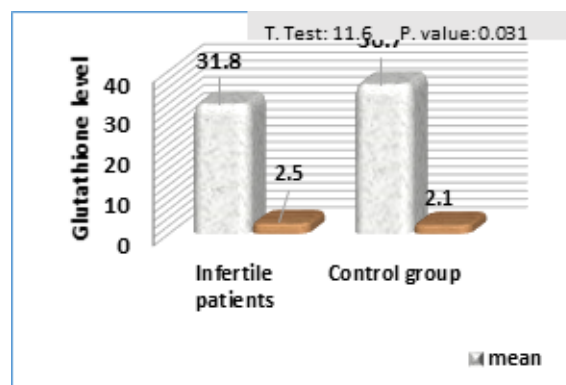


Figure 2: Level of GSH in infertile patients and healthy individuals

Discussion

The role of melatonin in reproduction in many species is related to seasonal reproductive cycles. In man, it has been demonstrated that changes in secretion of melatonin by the pineal gland can modulate the activity of the reproductive neuroendocrine axis⁽¹¹⁾. Kratz *et al.*,⁽¹²⁾ showed that abnormal sperm motility/ is associated with low melatonin levels. Sharbatoghli *et al.*,⁽¹³⁾ found that melatonin level was highly correlated with male infertility.

In consistence with our findings, Awad *et al.*,⁽¹⁴⁾ demonstrated that plasma melatonin levels in all infertile groups was reduced significantly compared with their levels in the fertile group. Additionally, Melatonin is considered to be a highly efficient free radical scavenger and an efficient antioxidant with its ability to cross all physiological barriers and to enter every cell and all subcellular compartments^(15,16). In correlation with our result, it has been reported that melatonin level in the semen and serum from infertile men is lower than that in fertile men⁽¹⁷⁾. The low level of melatonin in infertile men, in the present study, could still be defended in that; as the mean values of melatonin level show melatonin level ranges widely in individuals. On the other hand, the level of melatonin in human blood is affected greatly by varying numerous factors. Such factors include the amount of light, stress, body position, physical activity, time of the day and so on⁽¹⁸⁾. In general, it is accepted that a higher percentage of motile sperm is associated with improved fertilization rates and Monlloret *et al.*,⁽¹⁹⁾ has shown that the addition of melatonin to seminal samples can improve the overall motility and the percentage of progressively motile spermatozoa. Melatonin also appears to inhibit apoptosis in spermatozoa, with a reduction in early apoptotic events being demonstrated in human sperm thus prolonging sperm survival, these effects would serve to improve sperm quality, therefore increasing the probability of successful fertilization⁽²⁰⁾.

Infertility is associated with oxidative stress (OS), normally counterbalanced by different antioxidant systems⁽²¹⁾. OS has been recognized as one of the most important cause of male infertility⁽²²⁾. Malondialdehyde (MDA) is one of the final products of lipid peroxidation in seminal plasma⁽²³⁾. Toxic lipid peroxides are known to cause various impairments of sperm cells and may play a major role in the etiology of male infertility⁽²¹⁾.

Malondialdehyde (MDA) is an index of lipid peroxidation which may be a diagnostic tool for the analysis of infertility^(24,25). Kiziler *et al.*,⁽²⁶⁾ found that serum a glutathione levels of infertile group were significantly lower than their level in control group while MDA is higher in infertile men. Malondialdehyde can be used as a marker of oxidative stress and a potential marker in predicting assisted reproductive techniques outcome⁽²⁷⁾. The deleterious effect of free radicals on spermatozoa may be assessed by estimating malondialdehyde in seminal plasma and their relation with different sperm parameters⁽²⁸⁾. Patel *et al.*,⁽²⁸⁾ analyzed 22 fertile controls with 74 primary infertile males. Seminal malondialdehyde level was observed to be raised in all infertile groups except azoospermic cases in comparison to control. As well as, the study of Akbari-Asbaghet *et al.*,⁽²⁹⁾ showed that MDA level was significantly elevated in patients with infertility as compared with healthy persons. Sharma *et al.*,⁽³⁰⁾ showed that serum levels of glutathione peroxidase and albumin were significantly decreased in infertile men.

Chyra-Jachet *et al.*,⁽³¹⁾ showed that the activity of glutathione peroxidase (GPx) significantly lower in males with oligoasthenospermia compared with healthy volunteers and the level of MDA was significantly higher in males with as the no spermia than in the control group. Palani⁽³²⁾ also demonstrated an reduced level of glutathione and albumin in infertile men as compared with controls. Glutathione is the mother of all antioxidants, the master detoxifier and maestro of the immune system⁽²²⁾. It is one of the major endogenous antioxidant produced by cells participating directly in the neutralization of free radicals and reactive oxygen species, as well as maintaining exogenous antioxidants such as vitamins C and E in their reduced forms⁽³³⁾.

Conclusions

It was concluded that there were reduction in serum albumin, and glutathione, while elevation of MDA and melatonin in infertile men.

Conflict of Interest: None

Source of Findings: Self

Ethical Clearance: None

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Case Control Study: Estimation of Vitamin D Deficiency in Relation to Urinary Stones Formation among Tikritmale Population

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Abstract

Background: Renal stones is a common, highly recurrent disease with increasing prevalence worldwide. The association between vitamin D and calcium stones has often been investigated on the basis of the role of vitamin D in calcium homeostasis. Currently, there is no consensus on the management of vitamin D deficiency in patients with renal calculi, because of controversies about the relationship between vitamin D and calcium stones. However, the vitamin D deficiency is shown to be highly prevalent among kidney stone formers, and some studies found a higher prevalence in stone formers compared with non-stone formers. Mechanism for the association between vitamin D deficiency and calcium-based calculi according to the substantial role of inflammation and oxidative stress in calcium stone formation and also the pro-inflammatory effect of vitamin D deficiency. **Aim:** Evaluation the relation between vitamin D deficiency and renal stones formation. **Patients and methods:** This study is case control study performed from the 1st of November 2018 to 3rd of April 2019. The study included 100 male persons (50 cases of renal stone, and 50 persons control group (free renal stones but shared most other sociodemographic characteristics), sample was selected by convenience sampling method. Serum levels of vitamin D were done for both groups by boditech kit. The test uses competitive immunodetection method. **Results:** Results regarding the relation between level of vitamin D and renal stone formation show decrease serum level of vitamin D associated with renal stone (32%) in compare with the serum level of vitamin D in control group (12%), odd ratio (3.5). **Conclusions:** The study showed that the frequency of vitamin D deficiency increasing in patients with renal stones. Renal stones formation was more frequent among positive family history cases.

Key word: Vitamin D, Renal stone, Tikrit population

Introduction

Vitamin D comes from diet or dermal synthesis from sunlight, vitamin D is inactive biologically and a fat soluble vitamin. It regulates calcium absorption and its homeostasis. In humans, vitamin D₃ (cholecalciferol) and vitamin D₂ (ergocalciferol) is the most important compounds^[1]. In liver, vitamin D₃ is converted to calcidiol, 25-hydroxycholecalciferol. Vitamin D₂ is converted in the liver to (25(OH)D₂). It is widely known that circulating 25 (OH)D is the best indicator of vitamin D status^[2,3]. Then 25(OH) D₃ is converted in the kidneys into 1,25-(OH)D₃, a steroid hormone which is the active form of vitamin D and it acts as a hormone in the blood,

that regulate calcium and phosphate concentration in the bloodstream, that play a role in healthy growth and remodelling of bone^[4].

Vitamin D deficiency particularly (low serum 25-OH vitamin D) is a common health problem that can result from inadequate exposure to sunlight; malabsorption, or certain medications^[5]. Vitamin D deficiency may present as rickets or osteomalacia^[6,7], in addition to related problems as lower bone mineral density^[8], osteoporosis, colorectal cancer^[9], prostate carcinoma, congestive heart failure^[10], type 2 diabetes (insulin resistance), and depression^[11]. Vitamin D supplementation is associated with improvement of many health problems^[11]. Nephrolithiasis patients have low bone mineral

followed by items related to vitamin D deficiency and urinary stones. Data presented by suitable figures and tables, and analysis done by manual statistical methods.

Laboratory test: serum level of vitamin D was done for patients and control group by fluorescence immunoassay (FIA) for the quantitative determination of total 25(OH) D2/D3 level in human serum, the test uses a competitive immunodetection method^[3].

The cut-off (reference range)^[18]

25(OH)		Status
< 10 ng/ml	<25 nmol/L	Deficiency
10-30 ng/ml	25-75 nmol/L	Insufficiency
30-100 ng/ml	75-250 nmol/L	Sufficiency
-Working range 8.0-70 ng/ml -Conversion factor: 2.5×ng/ml= nmol/L		

Finding

The study included 50 patients with renal stone and 50 control (free of renal stones). Regarding demographic information of study groups, High percentage of patients and control were 20-40 years old about 20(40%), 21(42%) subsequently but there was not significant association at p-value<0.01 as in (table 1). so results showed that more frequent cases of renal stone were unemployed about 13(26%) and farmers were the less frequent about 2(4%). There was not significant association at p-value<0.01 (table 2).

The study included 82% of cases and 78% of control lived in the city (urban area). (figure 1).

Current study revealed that 40(80%) of patients were eat cheese, fish and milk for long period (figure 2). Figure 3 showed that 40(80%) of renal stone patients were drinking soft drinks frequently. Figure 4 presents that 18(36%) of cases and 5(10%) of control had positive family history of renal stone. The results found that 13(26%) of the patients have recurrent formation of renal stones (figure 5).

The study found that patients with renal stone had low serum level of vitamin D < 30 ng/ml about 16(32%) patients while only 6(12%) of control group had low serum level of vitamin D < 30 ng/ml. Odd ratio is 3.5 and the statistical analysis revealed significant association at

density^[12], high cardiovascular risk, and metabolic syndrome^[13]; therefore many studies suggest to give vitamin D to patients with renal stones history^[13]. Kidney stone formation occurs when urine contain high concentration of calcium, oxalate and uric acid crystals than the fluid, or when urine lack of substances which prevent crystals from sticking together, all these create an ideal environment for nephrolithiasis formation^[14]. Risk of urinary stones increased with increased dehydration^[15], eating high protein, sodium (salt) and sugar diet^[15], positive family history^[14], gastric bypass surgery, inflammatory bowel disease^[16], renal tubular acidosis, hyperparathyroidism, cystinuria, urinary tract infections, certain medications^[17].

Aim Study: Evaluation the relation between vitamin D deficiency and urinary stones among males in Tikrit city.

Objectives:

- 1- Identify age, residence and occupation of male patients with urinary stone in Tikrit.
- 2- Identify the associated factors with vitamin D deficiency.
- 3- Detect the relation between family history, and past history of recurrent urinary stone among patients with urinary stone.
- 4- Comparison of serum level of vitamin D in renal stone males patient and normal males in Tikrit city.

Patients and methods

Ethical and approval consideration: Permission was taken from patients to fill the information required and they were assured regarding the confidentiality of their responses.

Type of the study: This study is a case-control study that was carried out at the 1st of November 2018 until 3rd of April 2019. The study included 100 male persons 50 cases of renal stone, and 50 persons control group (free renal stones) but shared most other sociodemographic characteristics, type of food and soft drink intake, in addition to type of medications that increase risk of urinary stone formation as loop diuretics, sulfa medications, ciprofloxacin and others. Sample was selected by convenience sampling method. Data included demographic characteristics of sample,

p-value<0.01 between vitamin D level and renal stone as in table3.

Table1: Distribution of study group according to age

age	number of patients	percentage	NUMBER OF control	PERCENTAGE	ODD RATIO
LESS THAN 20	12	24%	10	20%	1.3
20-	20	40%	21	42%	1.6
40-	14	28%	16	32%	0.9
more than 60	4	8%	3	6%	1.0
total	50	100%	50	100%	--
<p>CHI SQUARE=0.48 DEGREE OF FREEDOM=3 P-VALUE=0.01 NOT SIGNIFICANT ASSOCIATION</p>					

Table2: Relation between occupation and study groups.

Occupation	Number of patients	Percentage	Number of controlling group	Percentage	ODD RATIO
Unemployed	13	26%	15	30%	0.8
Farmer	2	4%	4	8%	1
Employed	12	24%	12	24%	1
Business-man	12	24%	9	18%	0.7
Student	11	22%	10	20%	1.1
Total	50	100%	50	100%	
<p>CHI SQUARE=0.7 DEGREE OF FREEDOM=4 CORRELATION=0.1 P-VALUE=0.01 NOT SIGNIFICANT ASSOCIATION</p>					

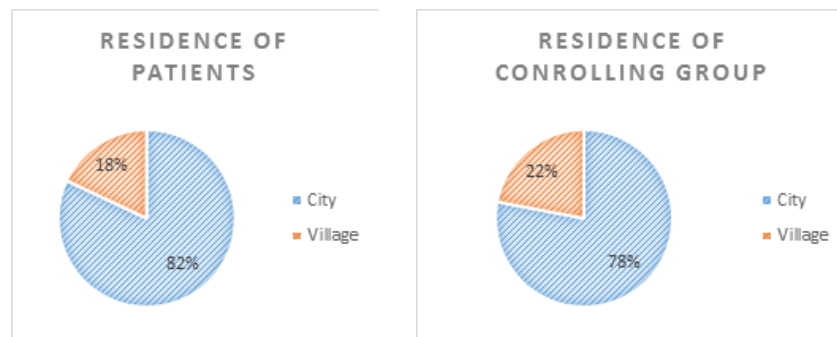


Figure 1: Relation between residence of study groups and urinary stone.

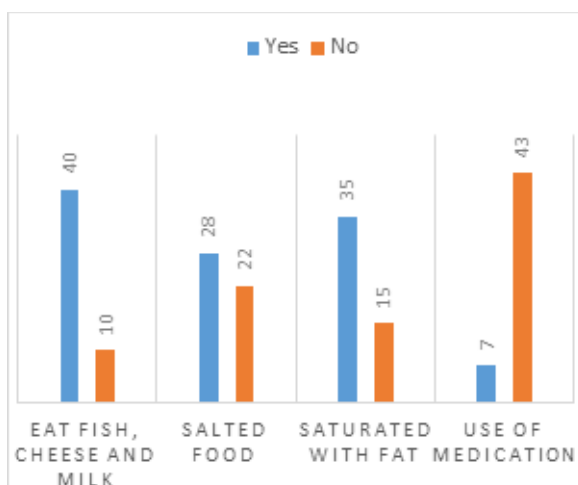


Figure 2: Relation between type food and medication among urinary stone patients

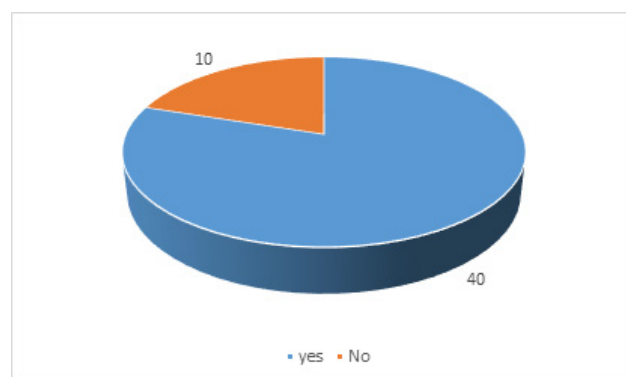


Figure 3: Relation between frequent drinking of soft drinks and urinary stone among patients

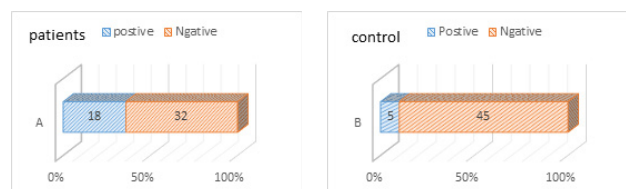


Figure 4: Family history of renal stones of study groups.

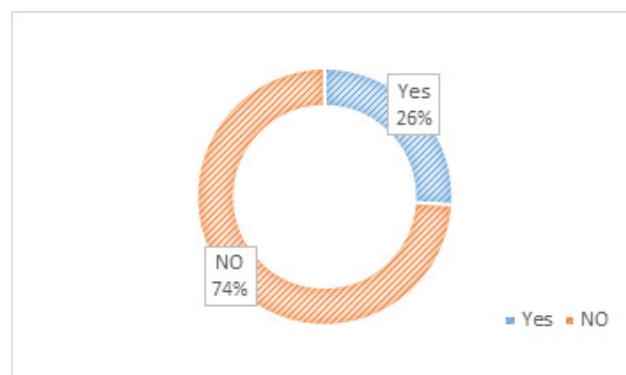


Figure 5: Recurrence of renal stones formation in the patients

Table 3 : The level of vitamin D among study groups

Serum vitamin D level (ng/ml)	Renal stone patients		Free renal stone persons		Odd ratio	
	number	percentage	number	percentage		
< 30 ng/ml	16	32%	6	12%		3.5
>=30ng/ml	34	68%	44	88%		
Total	50	100%	50	100%		
CHI SQUARE=5.82						
DEGREE OF FREEDOM=1						
CORRELATION=0.2						
P-VALUE=0.01						
SIGNIFICAN ASSOCIATION						

Discussion

The study was case control that evaluate the relation between vitamin D deficiency and urinary stones among males in Tikrit city as proved by odd ratio (3.5).

About patients of renal stone the peak mean of the patients was from (40) years which is agreed with Lieske article^[15], the control group samples chosen similar to ages of the patients to avoid bias.

For occupation, the results found that farmers had low frequency of renal stone about (4%) because the study was done in the urban area, also vitamin D investigation unavailable in general hospital therefore it was done in private laboratory where the farmer can't pay cost of investigations.

The most patients with renal stones (82%) live in the city and that because the sedentary life style and diet as preserved and fast food that increase the risk of renal stone formation^[18]. The positive family history of the patients with renal stone was (36%) of all patients with renal stone which agreed with other study which range the rate of family history between (17-37%)^[13], while in controlling group only (10%) of them have family history which indicated that the family history of the renal stone has a role in formation of the renal stones.

The recurrence of the stones in about 26% of the patients which less than the recurrence of other study which was (35-50%) of the patients ^[19].

Most the patients with renal stone (80%) were drinking many soft drinks frequently which containing oxalate that increasing the risk of formation of renal stone ^[19].

In the comparison between the patients with renal stones group and free control of renal stone show (32%) of patients with renal stone has below normal serum vitamin D level (<30ng/ml), while (12%) of control group have low serum level of vitamin D (<30ng/ml). Risk of renal stone formation increased among patients with low vitamin D level as proved by odd ratio (3.5), this may be because low serum level vitamin D increased parathyroid hormone levels, which cause hypercalcaemia this increase the risk of urinary stone formation^[20].

This research topic achieved for first time in the Tikrit city. There were obstacles that limit the sample size as unavailability of vitamin D investigation in general hospitals and cost of the investigation in private labs in the Tikrit city. In addition to absence of financial resources that support the study and its investigation.

Conclusions

1. Increasing the risk of vitamin D deficiency about one and half time in patients with renal stone (32%) than free renal stones (control group- 12%).

2. Urinary stone more frequent 36% among positive family history of the renal stone in comparison with control group 10%.

3. There were 80% of patients with urinary stone drinking of the soft drink frequently.

Recommendations:

1. Monitoring vitamin D level, parathyroid hormone and calcium level in patients with renal stone.

2. Further studies are needed to determine whether there is concomitant rise in intestinal absorption and urinary excretion of phosphate associated with vitamin D supplementation in relation to urinary stones.

Ethical Clearance: from research ethic committee in Tikrit university/college of medicine

Source of Funding : Self

Conflict of Interest : None

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Impact of Aspirin Suppositories on Fertility in Female Mice

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Abstract

The present study aims to investigate the effect of treatment with vaginal aspirin suppositories (Aspirin), with two different concentrations on the pregnancy outcome and the proportion of the sex ratio and some reproductive parameters such as the thickness of the lining of the uterus in female mice.

Guarantee the experimental work, the division (30) female mouse (10-12) a week and weight (32-52) grams in to three groups (10 mature females mice/group) including one group as control depending on different doses of aspirin suppositories (0.5 mg / kg, 1 mg / kg) and the administration period for 6 Day. The parameters were evaluated include, the study of pregnancy outcome, sex ratio, and histological changes in the uterus endometrial thickness using the pigments (hematoxyline and eosin stains).

The results of the current study, a significant improvement ($P < 0.05$) in the studied reproductive parameters using both of doses (0.5 mg / kg, 1 mg / kg) compared with the control group as: (pregnancy outcome, sex ratio, and histological changes in the endometrial thickness.

The results of vaginal dosing for the female mice also showed improvement in the standards mentioned above use high dose (1 mg / kg) of the same drug compared to vaginal delivery of low dose and control group. Therefore, it was concluded that the vaginal supplementation of aspirin probably has important role on the fertility and reproductive parameters and reduce the side effect of aspirin drug in females mice.

The present study showed clearly, an enhancement general numbers of embryos, as well as increases in male genders than control group.

Keywords: Mice, aspirin effect, ovaries, uterus

Introduction

The recurrent miscarriage, and lower pregnancy rates, and gender selection; are one of the reasons that are considered as indicators for fertility problems, and a large proportion were noted in patients with endometrial pattern (trilaminar and nontrilaminar); and thickness, and resistance index (RI). of the uterine artery, spiral artery or low uterine blood flow ^(2,1). Also, Women with ≥ 3 fetal losses and pregnancy is less, persistently positive results for endometrium is less than 7mm. thick; and does not occur when thickness is less 5mm ⁽³⁾.

Many studies, suggested that treating with aspirin improve the pregnancy rates increase uterine blood flow in women with unexplained infertility ⁽⁴⁾. Moreover;

wada *et al* ⁽⁵⁾ seen an improvement in the resistance of uterine flow, pregnancy rate and also lower rate of spontaneous abortion, in patients with low uterine flow, after aspirin treatment. However, others in this series, tried to determine if the addition of low doses of aspirin could improve the endometrial pattern and thickness. In our studies, this is the first and largest prospective series aiming to evaluate the role of aspirin in patients with thin endometrium. Low doses of aspirin; were associated with prolonged pregnancy and increased neonatal weight.

The use of intravaginal suppositories of aspirin has reduced the occurrence of aspirin side effects and aspirin allergy, also improve perfect environment for sperm movement across vaginal and cervix ⁽⁶⁾. Describe

the biochemical role in the production and effects of prostaglandin, its role in the prevention and treatment of poor pregnancy outcomes, as well as the possibility of congenital malformations, maybe improve sex gender and the other negative outcomes ⁽⁷⁾. The current study was intended to research an effect of vaginal aspirin suppositories on each of pregnancy outcomes, sex ratio and endometrial thickness.

Materials and Method

Experimental design

In the present work, thirty mature female mice were divided into three groups included one group as a control (each group include 10 mice), depending on different doses of aspirin namely low dose and high dose for 6 days of treatment.

Preparation

A local pharmacy prepared the vaginal aspirin suppositories as discussed by Tamer Guner et al⁽⁸⁾. Using completely a crushed one tablet (100mg), and

based on glycerogelatin base (based on displacement value, calculated quantity of micronized of glycerine and gelatine) by using standard fusion method, to obtain vaginal aspirin suppositories with different doses of aspirin (0.5mg/kg/day and 1mg/kg/day). Each dose administered for limited group of the female mice.

Histological study

Reproductive organs (uterus) of freshly scarified mice were fixed with formalin 10% for 12hr ⁽⁹⁾ and dehydrated through progressive increasing concentrations of ethanol alcohol, then cleared with xylene for 30 minutes, then replaced by other paraffin overnight in oven. Sections were made from paraffin block, then stained with alum haematoxylin and eosin stain. Slides were examined with light microscope using (4X). The parameter, endometrium thickness of uterus ⁽¹⁰⁾. Statistical analysis was performed using SPSS. Raw data analysis was performed using the Student's T-Test with Mean and Standard error of Mean Scores (S.E.M.) to allow comparison between pre- and post-treatment for all groups ⁽¹¹⁾.

Results

Table (1): Results of treated different doses of aspirin on total general number of embryos and male, female embryos of pregnant mice.

	Control group	(0.5mg/kg) aspirin dose	(1mg/kg) aspirin dose
Total number of embryos	6.44 ^A +0.346	7.600 ^B +0.257	9.701 ^B +0.395
Female embryos	3.210 ^A 0.11	2.400 ^A 0.010	2.300 ^A 0.121
Male embryos	3.230 ^A 0.024	5.200 ^B 0.206	7.400 ^B 0.142

Values mean + S.E.M.

Number of female mice per group = 10.

Similar capital letters means non-significant differences.

Different capital letters means significant differences.

In table (1), after treating with each aspirin dose (0.5mg/kg and 1mg/kg) for 6 days, a significant increment ($P<0.05$) was assessed in the amount of embryos as compared to the control group. Meanwhile, no significant differences ($P>0.05$) were noticed in female ratio when compared between control and treated group with each doses of aspirin for 6 days.

Additionally, significant increases ($P<0.05$) were recorded in male ratio in treated group of low and high dose for 6 days as compared to the control group.

There is evidence that a low dose of aspirin has a positive effect on pregnancy and that low doses of aspirin have been associated with prolonged pregnancy and have the effect of increasing the neonatal weight. Low thick endometrium, associated with poor uterine receptivity, and lower embryo implantation, has been demonstrated^(1,6). Likely, about for the patients with thin endometrium, aspirin administration was appeared to increase embryo implantation rate in animal study⁽⁷⁾, this is consistent with the results of tables (1and2). Weckstein *et al.*⁽⁸⁾ showed, that the recipient of the oocyte donation with a thin endometrium. got improved implantation rate and better pregnancy outcomes after the low-dose aspirin supplement. They also observed that the mechanism of aspirin is through improving the resistance of uterine flow by shifting local production of thromboxane toward prostacyclin. As it is displayed in a table (1), In women with unexplained infertility or uterine blood flow, aspirin has improved uterine flow and increases pregnancy rates and fetal weight^(4,12).

On the other hand; Mayor; *et al.*⁽¹³⁾ with the emergence of recent flow cytometric separations; With X and Y sperm and PGD, couples no longer have to adopt abortion to choose sex. (PGD) provides the only reliable way to determine the sex of offspring but this method is expensive. And it also requires the use of in vitro fertilization. We are probably think of the Shettles method, which is based on the premise that sperm with X and Y chromosomes have different characteristics and can affect the reproductive environment to prefer one or the other. May be, using aspirin in the form of vaginal suppositories, this reduces the side effects of the drug, and it is possible to provide an appropriate environment for sperm carrying the male genes, as showed in table (14,15)

Table (2): Results of treated different doses of aspirin on endometrial highest (μm) of pregnant female mice.

	Control group	(0.5mg/kg) aspirin dose	(1mg/k) aspirin dose
Endometrial highest (μm)	262.1 ^A +5.012	454.8 ^B + 3.125	576.2 ^B + 4.240

Values mean + S.E.M.

Number of female mice per group = 10.

Similar capital letters means non-significant differences.

Different capital letters means significant differences.

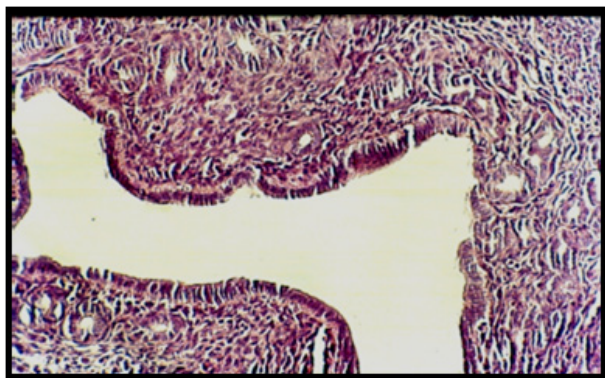
After 6 days of aspirin treatment in treated groups, table (2) shown significant elevation ($P<0.05$) in endometrial highest (thickness) in treated groups with both low dose and high dose of aspirin for 6 days of SC treatment as compared to the control group.

As for the effect of aspirin on endometrium difference trial thickness, as appear in table 2. several investigators have demonstrated statistical change in the endometrium after the aspirin significant supplement^(2,9). In finally,, higher pregnancy rate and better endometration. Aspirin therapy could be obviously increaserial pattern were achieved in the infertile patients with thin endometrium after aspirin administration respectively endometrial thickness or the resistance of uterine. Aspirin, which has an effect on cyclosogenase, has the ability to interfere with implantation and it also has the ability to support the maintenance of pregnancy, it was prescribed with increasing frequency to reduce the risk of mother thrombosis, the risk of miscarriage and poor pregnancy outcome. However, large studies confirm the relative safety of aspirin in low dose and overall positive effects on reproductive outcomes⁽¹⁶⁾. Thin endometrium associated with poor uterine receptivity and lower embryo implantation has been demonstrated^(17,18). As for the management of the patients with thin endometrium, aspirin administration was shown to increase embryo implantation rate in animal study⁽¹⁹⁾. Check; *et al.*⁽²⁰⁾ demonstrated the lack of positive impact of aspirin therapy on pregnancy rate after frozen embryo transfer.

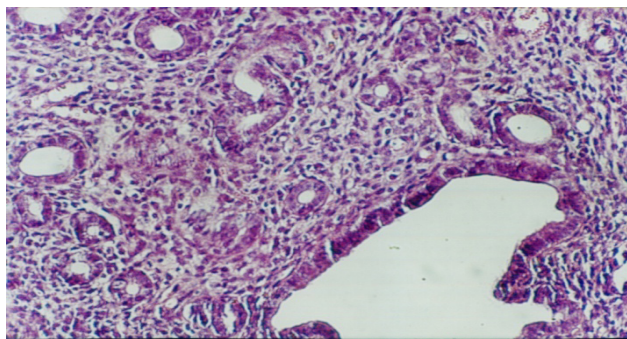
Effect of vaginal aspirin suppositories on uterus:

The histological sections of control groups (image1), shows normal histology. and no significant for any pathological features showed in the longitudinal uterus sections like congestion, atrophy, degeneration, and sloughing.

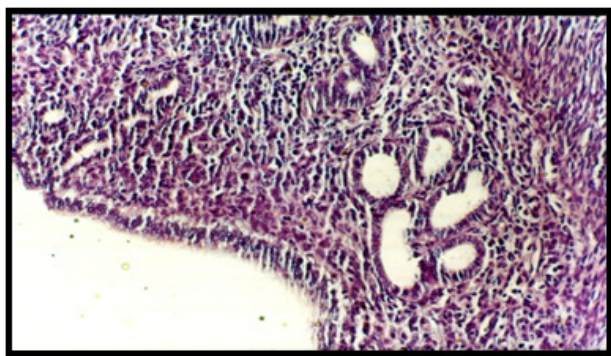
Similar observations were noticed in the histological sections of all treated groups for both of cell layers (image 2 and 3).



Image(1): section in uterine of a mature female mouse (control group) , showing the uterine endometetrail height (UL), (H & E , 200 X) .



Image(2): section in uterine of a mature female mouse treated with 0.05 mg / Kg /day of daily administration of the effects thevaginal aspirin suppositories for 6 days, showing an increase in the uterine endometetrail height(EH) (H & E , 200 X).



Image(3):Longitudinal section in uterine of a mature female mouse treated with 1mg / Kg /day of daily administration of theeffects thevaginal aspirin suppositories for 6 days,

showing increase in the uterine endometrail height (EH) (H & E , 200 X).

Conclusion

The present study showed clearly, an enhancement general number of embryos, as well as increases in male genders than the control group.

Also, significant elevation appeared in endometrial thickness in treated groups with both low dose and high dose of aspirin for 6 days of SC treatment as compared to the control group. The result suggested that the possibility of aspirin mechanism, is through improving the resistance of Uterine flow by shifting local thromboxane production towards prostacyclin, and these reflected in improving the uterine flow and increased the Pregnancy rate of women with unexplained infertility or uterine blood flow disorders.

Usingaspirin in the form of vaginal suppositories, this could reduce the side effects of the drug, and it is possible to provide an appropriate environment for sperm carrying the male genes.

Ethical Clearance: Nil

Conflict of Interest: Non

Source of Funding : Nil

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Detection of anti-rubella IgM, IgG and Antisperm Antibodies in the Sera of Aborted and Non-Pregnant Women in the Kirkuk City

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Abstract

Approximately 60% of cases of fetal loss from all repeated abortion conditions are unclear or attributed for some reason. This study was carried out in the Al Kirkuk general Hospital, from August to October 2017. A total of 148 females were screened for anti-sperm antibodies and anti-Rubella antibodies of IgM and IgG type by ELISA, 60 females were undergoing previous abortion (30 Pregnant + 30 Non-pregnant), and control group included 30 pregnant females without previous abortion, and 58 married without pregnancy.

The results compared with their corresponding data and statistically analyzed, the revealed the following:

The majorities 18 (41.8%) of study groups with positive anti-rubella IgG were between the age (26-35) years old, while minority 10 (7.1%) frequency of study groups with positive anti-rubella IgG were between the age (36-45) years old. The most of seropositive to anti-rubella IgG are from women whose non-pregnant but with previous abortion 20 (66.6%), Only 1 (3.3%) of pregnant women with previous abortion and pregnant but without previous abortion had seropositive to anti-rubella IgM, while 2 (6.6%) in non-pregnant women but with previous abortion.

Among these 58 cases about 16 cases was revealed elevated anti-sperm antibody ASA (27.6%). Out of 30 aborted 9 cases were high ASA (30.0%)

The sero-prevalence of ASA of study groups according to age groups, just 6 cases of non-pregnant married women (37.6%) of study groups with high ASA were between the age (25-35) years old, while among aborted married women 5 (55.6%) frequency of study groups with high concentration were between the age (25-35) years old.

Finally concluding that there is a high rate of susceptibility to rubella infection in women with childbearing age in Kirkuk City. Premarital screening for anti-rubella and vaccination of girls could minimize the risk of infection during pregnancy and childbearing period. ASA may be the cause of infertility in female and may be the cause in the increases of the rate of recurrent spontaneous abortion.

Key words: "Rubella virus", "antisperm antibody", fertility, abortion.

Introduction

Many organisms can cause the contagions from mother specially those had the ability to transmission *in utero* at different phases of the pregnancy^(1,2) inappropriate fetal outcomes such as recurrent abortions, infertility, deaths of fetus inside the uterine, still births, congenital deformities and other defects of reproductive system are associated with these contagions were documented

by prior studies^(3,4). The rubella virus cause the disuses "Rubella"⁽⁵⁾ known as "German measles"^(6, 7), from the family "togaviridae", where just about 50% of whole elementary "rubella" contagion are subclinical⁽⁸⁾.

Abortion, and congenital deformity these are the most serious consequences of "rubella" can be obtained from contagion in the period of the primary trimester for pregnancy⁽⁹⁾. Born infants

were developing congenital rubella syndrome (CRS) with percentage up to 90% from infected mothers in^(10,11). The major aim of inoculation plans against rubella is to remove or decrease infection in gravid females and the associated hazard of CRS⁽¹²⁾. Abortion result from different reasons, factors related to immune system are the most reported reason of miscarriage and the most significance ones are anti-sperm antibody (ASA)⁽¹³⁾. In gestation a critical function has been reported for ASA, there is a correlation between the rate of repeated unprompted miscarriage and the levels of ASA in serum of females. The production of ASA can be caused by a decrease in suppressor T lymphocytes⁽¹⁴⁾. The correlation of ASA with abortion are supported by many reports⁽¹⁵⁾. Production of ASA are directed against the antigens located on the sperm surface, In both women, and men, which have the ability to intervene with motility of sperm and transfer them through the reproductive tract of females, leading to acrosome and capacitates inhibition, impairment of fertilization, effect on the process of implantation, reaction, and the fetus growth and development impairment “ (ASA)” are IgG, IgA, and IgM immunoglobulin, detection of its can be occur in ejaculate, fluid of the follicular, mucus of the cervical, and serum⁽¹⁶⁾.

Methodology

This study was carried out in the Al Kirkuk general Hospital, from August to October 2017. A total of 148 females were screened for anti-sperm antibodies

and anti-Rubella antibodies of IgM and IgG type by ELISA techniques (direct and indirect), 60 females were undergoprevious abortion (30 Pregnant + 30 Non-pregnant), and control group included 30 pregnant females without previous abortion, and 58 married women without pregnancy. A complete information include (age, number of abortion, numbers of marriage year, history of abortion,) from all patients were recorded. Blood was collected by means of vein puncture with a sterile needle and syringe. Approximately three ml of venous blood was collected from each subject. The blood samples were centrifuged for 5 min. and serum were separated and stored in another plane tube at about (-20°C) until assayed. With avoiding repetitive freezing and thawing of serum sample.

Aims

This study was performed to detection of anti-rubella IgM, IgG and to determine the prevalence of anti-sperm antibodies in the sera of aborted and non-pregnant married women. Then study the correlation between the above parameters and the cause of abortion

Finding

Results in table (1) showed that the majorities 18(41.8%) of study groups with positive anti-rubella IgG were between the age (26-35) years old, while minority 10(7.1%) frequency of study groups with positive anti-rubella IgG were between the age (36-45) years old.

Table (1): Seropositive prevalence of anti-rubella IgM-IgG of study groups according to age.

Anti-rubella	Age groups in Years					
	18 -25		26-35		36-45	
	No	%	No	%	No	%
IgM	1	4.3	3	6.9	0	0
IgG	8	34.7	18	41.8	10	7.1
IgM-IgG	0	0	1	2.3	1	4.7

Ig= immunoglobulin, No= number, %= percentage.

Results in table (2) showed that the most of seropositive to anti-rubella IgG are from women whose non-pregnant but with previous abortion 20(66.6%), only 1(3.3%) of pregnant women without previous

abortion have seropositive to anti-rubella IgG.

Only 1(3.3%) of pregnant women with previous abortion and pregnant but without previous abortion had seropositive to anti-rubella IgM, while 2(6.6%) of non-

pregnant but with previous abortion women had seropositive to anti-rubella IgM.

Table (2): Seropositive prevalence of anti-rubella IgM-IgG of studied group.

Study groups	No.	IgM		IgG		IgM-IgG	
		NO.	%	NO.	%	NO.	%
Pregnant with Previous abortion	30	1	3.3	17	56.6	1	3.3
Pregnant without previous abortion	30	1	3.3	1	3.3	0	0
Non-pregnant with previous abortion	30	2	6.6	20	66.6	1	3.3

IgG= immunoglobulin G, No= number, %= percentage.

In this study about 86 subjects of female samples collected, nearly 30 sample were aborted and remain were just married without pregnancy. Among these 58 cases about 16 cases was revealed elevated anti-sperm antibody ASA (27.6%) and 26 (44.8%) of female was with low ASA. Out of 30 aborted female 9 cases were high ASA (30.0%).

Table (3) Distribution of study groups according to ASA concentration.

ASA level	Non aborted married women		Aborted married women	
	NO.	%	NO.	%
High. 62-250 U/L	16	27.6	9	30.0
Equivocal. 46-50 U/L	16	27.6	7	23.3
Low. <45 U/L	26	44.8	14	46.7
Total	58	100	30	100

ASA= antisperm antibodies, U/L= unit per liter, No= number, %= percentage.

Results in table (4) showed that just 6 cases of non-pregnant married women (37.6%) of study groups with high concentration of anti-sperm antibodies were between the age (25-35) years old, while among aborted married women 5 (55.6%) frequency of study groups with high concentration of anti-sperm antibodies were between the age (25-35) years old.

Table (4): The high concentration sero-prevalence of ASA of study groups according to age groups.

Age group (years)	Aborted married		Non aborted married		Total	
	High 62-125 U/L		High 62-125 U/L		NO.	%
	NO.	%	NO.	%		
15-25	3	33.3	5	31.2	8	32.0
25-35	5	55.6	6	37.6	11	44.0
35-45	1	11.1	5	31.2	6	24.0
Total	9	100	16	100	25	100

ASA= antisperm antibodies, U/L= unit per liter, No= number, %= percentage.

Discussions

The major infectious disease that affecting gravid women and their embryo “throughout the world is Rubella”⁽¹⁷⁾.

The present study outputs showed that 3(6.9%), 18(41.8%) were positive for IgM antibody and IgG respectively, when compared with others finding, regarding it is lower than which obtained by Adam et al., who documented that IgG antibodies against Rubella were detected in 95.1% among pregnant ladies, and in agreement with their result of insignificant association with age, most infections were occurs pervious the 35 old⁽¹⁸⁾. Where it is likely to the Asamarai et al report, about seroepidemiology of “Rubella” in “Kirkuk” city⁽¹⁹⁾. The overall anti-rubella IgG seroprevalence among pregnant women was (56.6%) in the present study, this result is almost like to another study conducted in Baghdad⁽²⁰⁾, while the percent it is less than other studies conducted elsewhere abroad^(21, 22) and higher than those reported by certain studies^(23, 24).

The positivity rate 66.6 % for anti- rubella IgG of the non –pregnant women in the childbearing age in the present study had been within the range obtained by other studies⁽²⁵⁾. The presence of an IgG after clinical or non-clinical infection with the rubella virus during the last pregnancy may be the reason for the discrepancy in the current study results, the rate of positive in non-pregnant women compared to pregnant women was the slightly higher (56.6% vs 66.6%), were positive for anti-rubella IgM in this study was 3.3% of controlled pregnant women⁽²⁶⁾.

Any defect to mechanisms of protective immunomodulatory and barrier of blood-testis may be result in the infertility with the self-immune causes. The study showed that of the 30 cases of abortion in 9 cases with a high concentration of ASA (30.0%), this was consistent with Dr. Karimi, who revealed that the antibodies to the sperm have a crucial role in pregnancy, and there is an association between antisperm antibody level in women serum and the rate of repeated spontaneous miscarage⁽²⁷⁾.

Fertility can be affected adversely by antisperm antibodies while normally controlling may be via the antibodies against idiotypic which along with the factors of immunosuppressor in semen that block their inducing to a significant degree⁽²⁸⁾.

In this study we had taken 58 infertile female Out of 16 infertile female 33.3% were having increasing ASA which is higher than a similar study conducted at USA⁽²⁹⁾, and reported an ASA prevalence of females it reached 4.8% with unexplained infertility, Other researchers reported the frequency of circulating ASA from 1.3% to 7.3% in serum of infertile women^(30, 31).

Conclusion

High rate of susceptibility to rubella infection in women with childbearing age in Kirkuk City. Premarital screening for anti-rubella and vaccination of girls could minimize the risk of infection during pregnancy and childbearing period. ASA may be the cause of infertility in female and may be the cause in the increases of the rate of recurrent spontaneous abortion.

Conflict of Interest: Non

Source of Findings: Self

Ethical Clearance: Nil

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Direct Molecular Detection of *Gardnerella Vaginalis* and *Lactobacillus Spp.* in Women with Bacteria Vaginosis

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Abstract

Bacterial vaginosis (BV) is considered to be the most frequent vaginal infectious disorder in women of childbearing age. The aim of this study was direct detect of *Gardnerella vaginalis* and *Lactobacilli* in vaginal mucus of suspected women. A total of 60 baseline vaginal swabs were obtained from 20 subjects with BV and 40 subjects without BV seen between March 2018 and November 2019. In this prospective study, participants attending private clinics in Kirkuk city-North of Iraq BV was defined using Amsel's clinical criteria for all subjects (with the presence of at least three of four criteria needed to establish a diagnosis of BV). In addition, Nugent criteria (Gram stain score ≥ 7) were done to evaluate BV status in all subjects attending the clinics. Vaginal swab samples were collected from each women in the study and stored for molecular detection of *Gardnerella vaginalis* and *Lactobacilli* by multiplex real time PCR. The presented study found that 80% of BV patients were suffered from previous abortion and 20% haven't this history while 7.5% of women without BV infection have history of abortion. In this study, 50% of women infected with BV were within the age group 30-39 year followed by the age group 20-29 years (35%) and 45% of women without BV infection were within the age group 30-39 year. In the present study, women with clinically diagnosed with BV were 90% positive by multiples RT-PCR, 2% were with mixed (*Gardnerella vaginalis* and *Lactobacillus*) and no pure infection with *Lactobacilli* was occurred BV positive women, while the study showed that 92.5% of BV negative women were *Lactobacilli* positive by multiples RT-PCR, 5% of them showed mixed positive by multiples RT-PCR and 2.5% were with *Gardnerella vaginalis*. The result was highly significant.

Keyword: *Prostate cancer; BK polyomavirus, BKPyV*

Introduction

Bacterial vaginosis (BV) is known as to function as most frequent genital infectious problem in women of childbearing time (1). Rate of 5-35% have been reported from European and American studies. The condition is symptomatic in half of the women and also represents a psychological burden (2). Prevalent clearly, this rattled vaginal microflora footing substitute wise sequelae such as non-gonococcal, non-chlamydial PID (Pelvic Inflammatory Disease), postpartum endometritis and preterm birth (3). Actually, 40% of the cases of natural preterm labor and preterm opening are posture to be connected around BV. This impair of *Lactobacillus* associated down the overgrowth of anaerobic and microaerophilic bacterial bring forth defines bacterial vaginosis (4). By making an abnormal foul vaginal discharge, BV need

a sway with respect to women's sexual connections and caliber of life (1). Moreover, BV will be joined with pelvic incendiary illness Furthermore more terrific powerlessness will sexually transmitted infections (2). BV-associated microscopic organisms bring been identified with expanded danger about preterm conception and unfriendly neonatal results (6). Those research center technique by acknowledged Concerning illustration the gold standard for analysis for BV will be microscopy of Gram-stained vaginal smears (3). In light both the Nugent scoring framework and the Amsel criteria utilized within clinical act bring their limitations, DNA-based assays focusing on the microscopic organisms connected with BV need aid continuously wailing to objective, reproducible, and exact analysis of the BV state (7). BV may be distinguished Likewise An polymicrobial disease, Yet determination of the way pathogenic microbial species Also strain variants

included will be at present ongoing (2). Vaginal anaerobe, *Gardnerella vaginalis*, would recouped from the vaginal tests for Practically the greater part ladies with BV. *G. vaginalis* possesses a number about virulence variables including handling for sialidase a and the poison vaginolysin(6). It also has the ability to stick should vaginal epithelial units What's more secure a biofilm(2). In spite of the fact that *G. vaginalis* may be connected with different clinical conditions, it need been found for vaginal specimens for solid individuals, albeit frequently all the done easier numbers over for BV cases(7). Its great referred to phenotypic What's more hereditary heterogeneity prompted those advancement from claiming genotyping schemes dependent upon examination for entirety genome successions What's more cpn60 genes(1). So the aim of this study was direct detect of *G. vaginalis* and *Lactobacilli* in vaginal mucus of suspected women.

Material and Method

A total of 60 baseline vaginal swabs were obtained from 20 subjects with BV and 40 subjects without BV seen between March 2018 and November 2019. In this prospective study, participants attending private clinics in Kirkuk city-North of Iraq BV was defined using Amsel's clinical criteria For all subjects (with those vicinity for no less than three for four criteria required will establish An finding from claiming BV). Over addition, Nugent criteria (Gram stain score ≥ 7) were carried on assess BV status on all subjects going to the centers.

Sample collection

Every subjects underwent speculum examination with accumulation for vaginal liquid to saline microscopy, KOH preparation, pH measurement, and evaluation to amiiidae smell. Will get examples to those execution for PCR assays, a polyurethane froth swab (Catch-All; Epicenter) might have been brushed against those parallel vaginal wall, resheathed, and solidified

until those DNA extraction step.

DNA extraction.

Vaginal swabs for bacterial PCR were placed in 15-ml conical vials with 2 ml of saline and vortex mixed for 1 min to dislodge cells. The sample solution was centrifuged at 14,000 rpm for 10 min, and the pellet was resuspended in 100 μ l supernatant. DNA was extracted from the pellet using the Ultra Clean Soil DNA Kit (Zymogen) according to the manufacturer's instructions.

Gardnerella vaginalis/*Lactobacillus* specific RT-PCR assays.

"*Gardnerella vaginalis*/*Lactobacillus* specific PCR assays" (Sacace biotechnology, Italy) is based on two major processes: "isolation of DNA from specimens and multiplex Real Time amplification". "Amplification results of *Gardnerella vaginalis* are detected on the Fam/Green channel, amplification results of *Lactobacillus* spp are detected on the Joe/HEX/Yellow channel". For quantification of the DNA of "*Lactobacillus* spp and *Gardnerella vaginalis* all calibrators should be used and defined as standards with specific concentrations". "Fluorescence is detected at the 2nd step of Cycling 2 stage (60 °C) in FAM/Green and JOE/Yellow/Hex/Cy3 fluorescence" channels. *Lactobacillus* spp. is detected on the JOE(Yellow)/HEX/Cy3 channel, *Gardnerella vaginalis* on the FAM (Green) channel

Statistical Analysis

Computerized statistically analysis was performed using Mintab ver 18.0 statistic program. Comparison was carried out using Chi-square (X2) for determination of the P. value.

Findings

The presented study found that 80% of BV patients were suffered from previous abortion and 20% haven't this history while 7.5% of women without BV infection have history of abortion, Table 1.

Table 1: Relation of BV infection with history of abortion.

History of abortion	Patient with BV		Patient without BV		P. value
	No.	%	No.	%	
Yes	16	80	3	7.5	< 0.01
No	4	20	37	92.5	
Total	20	100	40	100	

In this study, 50% of women infected with BV were within the age group 30-39 year followed by the age group 20-29 years (35%) and 45% of women without BV infection were within the age group 30-39 year, Table 2.

Table 2: Relation of BV infection with history of abortion.

Age groups (years)	Patient with BV		Patient without BV		P. value
	No.	%	No.	%	
20-29	7	35	12	30	> 0.05
30-39	10	50	18	45	
40-49	3	15	10	25	
Total	20	100	40	100	

In the present study, women with clinically diagnosed with BV were 90% positive by multiples RT-PCR, 2% were with mixed (*G. vaginalis* and *Lactobacillus*) and no pure infection with *Lactobacilli* was occurred BV positive women, while the study showed that 92.5% of BV negative women were *Lactobacilli* positive by multiples RT-PCR, 5% of them showed mixed positive by multiples RT-PCR and 2.5% were with *G. vaginalis*. The result was highly significant, Table 3.

Table 3: Detection of *Gardnerella vaginalis*/*Lactobacillus* in women with positive and negative BV.

Gardnerella vaginalis/Lactobacillus (RT-PCR)	Patient with BV		Patient without BV		P. value
	No.	%	No.	%	
Gardnerella vaginalis	18	90	1	2.5	< 0.01
Lactobacillus	0	0	37	92.5	
Mixed (<i>Gardnerella vaginalis</i> and <i>Lactobacillus</i>)	2	10	2	5	
Total	20	100	40	100	

Discussion

Advancement of a normal device for those analysis about BV may be critical, Since there is an absence of reliable, standardized procedures, Furthermore target symptomatic instruments (3,5). Those Amsel criteria and the NS need aid the 2 at present accessible symptomatic techniques and are regularly utilized On distributed investigations. However, they are not routinely utilized Toward Doctors. The study demonstrated that 92.5% of BV negative women were with *Lactobacilli* sure Eventually positive by RT-PCR, 5% from. In the this study, those rate of *lactobacillus* spp. were those predominant in vaginal canals of women without BV infection which might have been in understanding with past finding (8). This discovering might have been an pointer for sound vagina in any case its vicinity for low sums with a secondary extent from claiming different

pathogens will be pathogenic. They assume a paramount part in securing against pathogens intrusion or excess by processing for hydrogen peroxide, bacteriocins, and lactic acid (9). In this study, half for women suffered from BV were in age 30-39 year, followed by 20-29 year (35%). AL-Aouadi (10) exhibited that the age class (20-29) & (30-39) were affected with higher recurrence rate of BV (28. 26% and 36. 95 % respectively). This may be expected will helter skelter rate of pregnancy Also change in the ordinary vaginal greenery over which the typically predominant *Lactobacilli* would swapped by pathogenic microscopic organisms. AL-Aouadi (10) additionally indicated that the recurrence for *G. vaginalis* distinguished by PCR system which might have been (45. 65 %) on and (36. 6 %) in the control group. The finding of BV is normally settled on clinically, practically ordinarily At no less than three crazy of four Amsel criteria are present, including a

thin, homogeneous, smooth vaginal discharge; vaginal-fluid pH more excellent over 4.5; An certain whiff test (i. E. , generation of a fishy smell At 10% potassium hydroxide will be included with a slide holding vaginal fluid); What's more piece of information units (>20% from claiming epithelial units for undefined outskirts because of follower bacteria) once minute examination for vaginal liquid (1). However, some patients without BV may manifest clinical findings similar to those of BV, such as those with *Trichomonas vaginalis* vaginitis, in which subjects may have vaginal discharge, elevated vaginal pH, or a positive whiff test (3,6). Janulaitiene et al (11) exhibited that *G. vaginalis* was detected by PCR in most samples of BV women. Real-time PCR has been applied previously for the description of changes in the vaginal microflora. Zariffard et al (12) and Sha et al (13) used real-time PCR on frozen cervicovaginal lavage samples to quantify the presence of *Mycoplasma hominis*, *G. vaginalis* and the combined presence of *L. crispatus* and *L. jensenii*, in comparison with the microscopic interpretation of the Gram stain. Bradshaw et al. (14) applied real-time PCR for *A. vaginae* for a follow-up study of recurrent BV before and after treatment with oral metronidazole. Ferris et al. (15) applied real-time PCR for *G. vaginalis* to samples of six BV patients before and after treatment with a topical metronidazole gel. Thus far, only one group, studying advanced dental caries, used real-time PCR to quantify the presence of specific *Lactobacillus* species (16). Rampersaud et al (40) was found only in *Lactobacillus* iners, a common constituent of the vaginal microflora. No cross-reactivity was detected against non-*G. vaginalis* species, while the PCR fragment was detected in all *G. vaginalis* isolates tested including species from ATCC. The bacterium specificity of the assay was confirmed by sequencing the PCR product.

Conclusions

G. vaginalis was the most detected pathogen in BV women and in its may occur also in non-BV women.

Conflict of Interest: Non

Source of Findings: Self findings.

Ethical Clearance: This research was carried out with the patient's verbal and analytical approval before the sample was taken. According to this approval, all the samples were collected and the tests were carried out. A copy of the results of the tests was then given to

the patients.

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Intra-Articular Hyaluronic Acid Injection Versus PRP Injection Regarding Cost-Effectiveness in Treatment of Osteoarthritis of the Knee (OAK) in Salah- Aden province

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Abstract

Background: Osteoarthritis of the knee (OAK) , that may be caused disability and decreasing the quality of life. Intra-articular injections can be provide relief from symptoms as platelet rich plasma(PRP) and hyaluronic acid (H.A)injection. Aim of current study is examining the cost-effectiveness of both (PRP and H.A) injection products in treatment the Osteoarthritis of the knee (OAK) . The cost is a very important issue in areas where there is a large number of displaced people. **Patient and Method:** 90 Patients with OAK ,with age range (38–65) years, with a symptom of more than 1 year, diagnosis by X-ray (the Kellgren–Lawrence grade from 1– 4) , also done.45 patient given a hyaluronic acid injection and 45 patient given PRP the patient selected according to their socioeconomic status(patient who is financially capable were given a hyaluronic acid injection. The hyaluronic acid injection cost about 200\$ while PRP cost 25\$. the study was carried in a private clinic , the period from January 2017 to January 2018. The patient selected from people of Salah Aldin province were a large number of them were displaced from their places and were financially incapable.**Results:** When compared, hyaluronic acid and PRP injection in OAK joint there were(non-significant difference) regarding clinical improvement ,and outcome but the important difference is the cost of the injection (average 200\$ for hyaluronic acid injection and 25\$ for PRP injection) which significantly important regarding cost-effectiveness in areas of low financial income (special concern for areas where displacement occurred).**Conclusion:** results of current study showed that using PRP in OAK is more cost effective in areas of low income.

Key words: *hyaluronic acid ;cost-effectiveness ; osteoarthritis ; knee; platelet rich plasma.*

Introduction

Osteoporosis (OAK) is a chronic progressive disease which affects in people (≥ 45 years) percentage (20%) of According to a survey of causing lost working time in United States, OAK is most common caused losing performance work after pain in low back ⁽¹⁾. feacher by low bone quality and low mass of the bones (density) ,espatiey in olde people, In most of the cases, osteoporosis is asymptomatic (silent disease) the condition usually appears after bone fracture only.⁽²⁾

With increasing in life, expectancy, its estimating that need for knee joint surgery will increased more than (6) times by years 2030, cause significant economics

burden for pain control, and patient rehabilitation.⁽³⁾

The goals targets of treatment OA are increased functions and mobility, reduced pain; and slower disease progression ; prevention or correction of deformity, there are many conservative knee maintenance treatments that has short term efficacy and has advantages as well asdisadvantages ^(4,5). Such as intracranial corticosteroids and nonsteroidal antiinflammatory drug(NSAIDs)are commonest using for arthritis.treatments ,Despite their easy access and lowcost, this treatments, have systemic adveses effect and may causing

desraction of joint cartilage(J.C) and flare up the process of osteoporosis.^{(6,7).}

In recent years, due to the high costs of OAK management, effective treatment options in tissues healing has taken into consideration for preventing development of OA^{(8).}

The PRP are autologous therapy, that included plasma for patients, which contains growth factor(G.F) from platelets and internal fibrin scaffold(F.S). rationales for used PRP are stimulating natural healing chain also regenerate tissue by releasing “supraphysiologic” directly from platelet-deriving factors into the treatment siting.^{(9,10).}

Most previous studies believe that PRP therapy has concentration of platelet (4-6)times higher than full blood (200000mm^{-3}). Is it possible to be reversed or ineffective to suppress healing process. classified PRP into 4categories, depending on the contents of: leukocyte-; pure_ ; plasma _ (rich plasma) ^(11,12).

Applications of biological treatment suchas (PRP) in musculoskeletal disordersgrow significantly. Despite the comparison (PRP) with otheres intra.articular; softtissue injections within the joint leding to conflicting results, PRP appears to have beneficial effects on healing and functional improvement of infected tissue^(13,14,15)

So,Hyaluronic acid (HA) has high M.W, Which includes a repeated unit of D-glucoronic-acid and acetylglucosamine synthesized by fibroblasts; chondrocytes and synoviocytes. It is available in extracellular matrix and synovialfluid , and its responsible for lubricant and viscoelastic charecterstic of, synovial fluid^{(16,17).}

Normal of adult knee joint have synovial fluid(2ml), thatcontaining about (2.5-4mg /ml) ofHA, has an average (M.W) of $5 \text{ to } 7 \times 10^6$ k.D. In the the O.A setting,both molecular weight and internal HA concentration decrease due to dilution of secondary synovial fluid for effusion, abnormal produing of synoviocyte, as well as molecular fragmentation^{(18).}

Although intra-articular injection mechanism of H.A in improved the O.A symptoms was clearly unknown, it appears to have many roles in joint mechanical supporting and its effects metabolic,that cause HA synthesis, synthesis of matrix cartilage components,

stimulated metabolism of chondrocyte, inflammatory process cartilaginitive metabolism, as well as inhibition of chondrodegenerative enzymes ^{(19,20).}

Patient and Method

Ninty OAK Patients (criteria ofAmerican College of Rheumatology) ,with rengen of the age from (38–65) years, with more than 1 year symptom , diagnosis X-ray(Kellgren_ Lawrence grade from 1–4) also done.42 patient given a hyaluronic acid injection and 48 patient given PRP the patient selected according to their socioeconomic status(patient who is financially capable were given a hyaluronic acid injection. The hyaluronic acid injection cost about 200\$ while PRP cost 25\$. the study was carried in a private clinic , the period from January 2017 to January 2018. The patient selected from people of Salah Aldin province were a large number of them were displaced from their places and were financially incapable.

Exclusion criteria included historyof immunodeficiency ; diabetes mellitus(D.M) ; malignant disorders ; autoimmune and platelet disorders ; ctive wound or infection in knee area ; severe trauma toknee, treatment by antiplatelet and anticoagulant medications(tendays) before injection, using systemic corticosteroids (two weeks) before the PRP injections; using NSAIDs (twodays) before injection; allergyto avian proteins; hypersensitivity to hyaluronate; feathers and products egg.

History taking for all participants; test laboratory including (C.B.C with differential; C.R.P; E.S.R); physical examination; knee radiography(Anterior-posterior “AP” standing alsolateral views), as well as a survey of using medications and supplements were done to all patients.

Outcome measures

Questionnaire of WOMAC (Western Ontario and McMaster Universities Arthritis)index for the evaluation of patient’s function through the interview conducted. Each of these (8) domains is recorded from (0 - 100) with fewer points that indicating further disability.

The Questionnaire of WOMAC is a widely using tool in arthritis studies which including(5) items of pain,(2) for sclerosis, and (17) for function evaluation,Each thequestion was recorded scored (0 to5) lower points indicating less pain and better functional condition.

Participants were followed 4;24 and 48 week after the treatment. So during these period, they are assesse for : swelling ;analgesicpain and stiffness.After twelve months, the(WOMAC) forms were fulfilled again.

Data analysis : performed by used Student-t-test for difference between 2 independent means ; Chi-square test used for qualita

tive data as well as for the difference between 2 dependent means (Baseline Vs Week 48) used Paired-t-test (conceded $P \leq 0.05$ as statistical significance).

Finding

Current study included 90 subjects, women and men , with mean age (58.79) years . PRP group as 48 subjects and HA group as 42 subjects(table 1).

Table(1) ditrbution of study groups (PRP and HA) according to gender

Gender	PRP group		HA group		P value
	No	%	No	%	
Female	30	62.5	28	66.7	0.848
Male	18	37.5	14	33.3	
Total	48	100	42	100	
WOMAC	(Mean ± SD)				
Stiffness	2.25 ±1.77		1.89 ±1.71		0.331
Pain	8.48 ± 4.20		6.10 ± 3.83		0.071
Phys. Function	28.92 ±12.65		19.88 ± 12.32		0.001*
*Significant (0.05 level).					

Figure (1) Mean WOMAC Index Score

Table(2): Scores of WOMAC index during period study

WOMAC Index Scores	Stiffness	Pain	Phys. Function	Total
PRP group				
Baseline	2.2 5 ±1.78	8.47 ± 4.19	28.93 ± 12.66	39.60 ±17.08
Week (48)	1.20 ± 1.5	4.04 ± 3.40	13.20 ±10.40	18. 45 ± 14.36
Change (48w <i>Vs</i> Baseline)	1.05±1.78	4.39±3.57	15.77±10.80	21.11±14.18
P value	0.0001*			
HA group				
Baseline	1.90 ±1.74	6.92 ± 3.83	19.90 ±12.33	28.70 ±16.71
Week (48)	2.14±1.66	5.10 ±3.73	19.54 ±11.11	27.48 ±16.37
Change (48w <i>Vs</i> Baseline)	0.25±2.11	1.84	0.3±13.69	1.22±18.65
P value	0.0001*			
*Significant (at 0.05 level).				

Figure (2) Mean WOMAC Index Score PRP**Figure (3) Mean WOMAC Index Score HA**

Primary analyses showed that WOMAC meaning that factor of pain was significantly reduced (2groups) after (48.weeks) of follow-up, also these reductions were higher than group of PRP group.

The stiffness; and total WOMAC meaning; physical functions are only improved meaningfully in (P.RP) group, as shown in fig. (2&3).

Both groups showed the significant improvement in patients symptoms regarding stiffness; pain; and the physical functions, cost of PRP is 25\$ while the cost of HA is 200\$.

Discussion

The need for the cost-effective treatments to the OAK, is very important because of increasing incidence and prevalence of disease, creating an increasingly large burden. With the higher initial TKA cost and High cost of the T.K.A revision, cost of effective treatment may delay the for thearthroplasty helps reduction (alleviate) some of economic burden imposing by OAK^(9,13,21).

Results of Roy *et al.*, 2014 showed effects of intra-articular PRP injection in OAK comparing with HA in a systematic review, So these study showed PRP resulted in improvement of function in all patients with pathology of knee, Compared to patients who received HA, PRP patients group had longer improvement. As well as better outcomes among these patients with milder forms of O.A than those advancing⁽²²⁾. These results were also consistent with the results of Alessandro *et al.*, 2018 in Italy (Bologna)⁽²³⁾.

Results of Kon *et al.*, 2011 conducted compared HA with PRP, with low M.W and HA with high M.W in one hundred and fifty patients⁽²⁴⁾. Treatment efficiency was assessed using by using visual analogue scale (V.A.S) and questionnaires of IKDC, At first therapeutic (2 and 6), months later months later, It showed great improvements in all parameters in (3) groups, Patients in group of PRP were more satisfying than both 2 groups. At the end of 2 months, also L.W.H.A and P.R.P group showing the improvements are similar and more than the H.W.H.A group. Whilst, Six months later, the PRP Group achieved better result. The improvement degree was related to the intensity of organic farming, so these increased improvement were achieved inpatient with

grade(0), O.A of these who had a grades(1;2;3) O.A⁽⁸⁾. In these study, patients appearance improvement in the PRP group over HA during the followup.

In 2013, report by Vaquerizo *et al.*, ninty six patients from 2 groups were given (3) session of PRGF injection or (1) session of injection HA, followed for (48) week, The PRGF efficiency was reducing in pain; stiffness as well as improved physical performance higher than HA⁽²⁰⁾. responses Patients to (PRGF) in all grades included: physical performance; stiffness; pain in WOMAC; OMERACT-OARSI and Lequesne were also pronounced more than HA, ²⁴ which were consistent with this study.

Conclusion

In our study we showed the effectiveness of both H.A and PRP usage for O.A of the knee regarding its cost, no other study showed the cost effectiveness in low income regions (areas of displaced people) which is very important when dealing with patient with this common health problem.

Conflict of interest: Non

Source of findings: Self

Ethical clearance: nil

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