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Correlated Levels of Anti-Inflammatory Interleukins (IL-4 and IL-10) with Allergic Conjunctivitis (AC) in Iraqi patients

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Abstract

Background Allergic conjunctivitis (AC) is caused by an allergic reaction, most common form was hypersensitivity reaction (type 1), that caused to perennial or seasonal AC. Current study was done in the Hospital of Ibn-AL-Hythem for eyes infections in Baghdad from September- August of 2018, one hundred and thirty-two serum were collected from allergic conjunctivitis patients after diagnostic by a specialist ophthalmologist according to have symptoms, who included 60 male and 72 female, with range age (15-51) years. Used the enzyme-linked immunosorbent assay (ELISA kit) from (Pepro-Tech Company , UK) for estimating levels of both Interleukins (IL-4 and IL- 10) in patients serum **Results** current study showed higher significant in age group (≥ 35 years) as percentage (57.6%) compare to age group (25- 35) as (28.1%) , also significantly higher in females (54.5%) than males (45.5%) , also level of IL – 4 in allergic conjunctivitis patients was (23.91± 2.09) compare to control (21.02± 2.99) ,so significantly increased levels of IL-10 in allergic conjunctivitis patients (14.99 ± 2.30) than control (11.10± 1.99).**conclusions** current study were **concluded** higher significant of allergic conjunctivitis Iraqi patients in age group (≥ 35 years), and the AC in females higher than males, as well as level of IL – 4 in allergic conjunctivitis patients were significant with control, and significantly increased levels of IL-10 in allergic conjunctivitis patients compare to control .

Keywords *: interleukins(IL-4; IL-10) ; allergic conjunctivitis(AC) ; Iraqi patients*

Introduction

Allergic conjunctivitis(AC) (or name as "allergic rhinoconjunctivitis," was most commonest allergic eye disorder, in the last decades in Iraqi patients , so allergic diseases were increased dramatically [(1)] Allergic eye usually are associated with others allergic conditions, such as dermatitis (atopic eczema) and allergic rhinitis (hay fever) , so th causing of eye allergies are similar to the allergic rhinitis (hay fever) and allergic asthma [(2)].

Allergic conjunctivitis is type of IgE-mediated hypersensitivity a wide term that recognised as 6 types including perennial allergic conjunctivitis (PAC); seasonal allergic conjunctivitis (SAC), contact lens-induced; atopic keratoconjunctivitis (AKC) drug-induced, and vernal keratoconjunctivitis (VKC)[(3)], However, both (VKC) and (AND) have pathophysiological and clinical pathophysiological characteristics completely different from (PAC) and (SAC), although some common markers of allergy[(4)]. AC common manifests as itchy, red eyes, or watering which included the symptoms of ocular symptom scores[(5)].

Cosmetics and Medication can play important role in causing of eye allergies , Any types of irritant, whether infectious, environmental, or manmade, can cause symptoms that consistent with an allergic of an eye , AC is caused by allergen-induced inflammatory response(AIIR), that allergen (such as animal dander ; pollen; and other environmental antigens[(6)]will be interacting with antibody (IgE bound to sensitized mast cells) causing clinical ocular allergic expression(COA). The pathogenesis of (AC) is predominantly an IgE-mediated hypersensitivity reaction. Activation of mast cells WHICH induces enhanced tear levels of the histamine; leukotrienes ;prostaglandins and tryptase, and this early response lasts clinically(20 minutes

–30 minutes) [2]. also, the degranulation of Mast cell to induces activated the vascular endothelial cells, that turn the expresses chemokines and adhesion molecules such as vascular cell adhesion molecules (VCAM) and intercellular adhesion molecules (ICAM) , so the other chemokines secreted [2] .

Many cytokines that derived from Th1, such as IL(2, 3) and IFN-γ, that mediates recruitment of the macrophages, while the cytokine that derived from Th2, such as IL (4 and 5), that participates in both chemotaxis and activation of the eosinophils[7], therefore aim of current study to assess levels of cytokine interleukin 10 (IL-10) and IL-4 in allergic conjunctivitis(AC) patients.

Material and Method

Sample collected :

Current study was done in the Hospital of Ibn-AL-Hythem for eyes infections in Baghdad from September - August of 2018. One hundred and thirty-two serum were collected from allergic conjunctivitis patients after diagnostic by a specialist ophthalmologist according to have symptoms such as Redness in inner eyelid or white of eye ; Itchy eyes; Blurred vision; Increased amount of tears ; and Swelling of the eyelid (fig.1) , who include 60 male and 72 female, with range age (15-51) years.

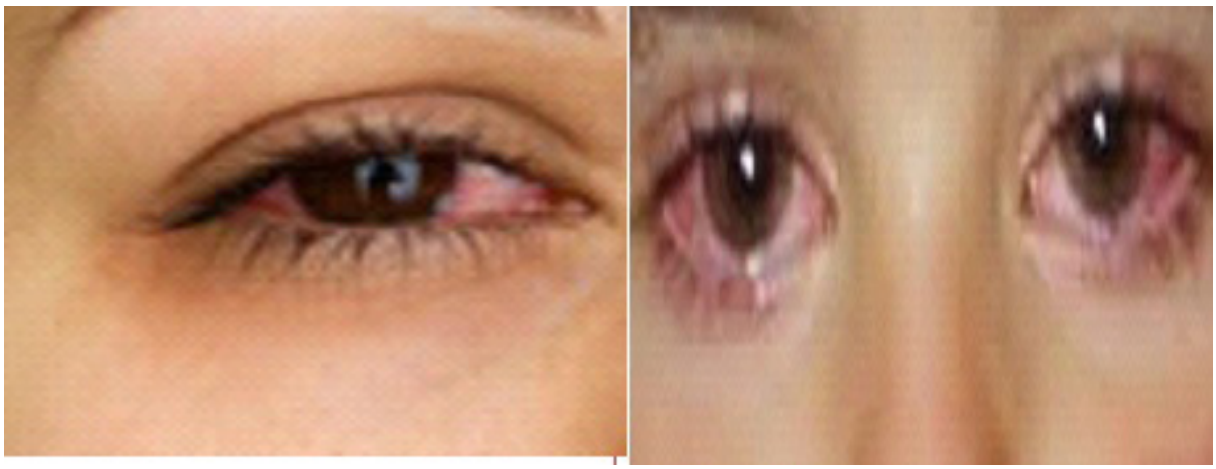

Figure (1): Symptoms of allergic conjunctivitis

Estimation levels of Interleukins (IL-4 and IL- 10) in patients serum

Used the enzyme-linked immunosorbent assay (ELISA kit) from (Pepro-Tech Company , UK) for estimating levels of both Interleukins (IL-4 and IL- 10) in patients serum according to the manufacturer's instructions.

Findings

Table (1): Distribution of allergic conjunctivitis patients according to age groups and gender

Age groups (years)	Patients		Control	
	No. = 132	%	No.= 50	%
≤ 25	19	14.3	12	24
25- 35	37	28.1	20	40
≥ 35	76	57.6	18	36
Total	132	100	50	100
Gender				
Male	60	45.5	25	50
Female	72	54.5	25	50
Total	132	100	50	100

Results in table (1) showed the range age of allergic conjunctivitis patients was (15-51) years, also Majority of allergic conjunctivitis patients within age groups of (≥ 35)years as percentage (57.6%) compare to age group (25- 35) as (28.1%) , also significantly higher in females (54.5%) than males (45.5%) . These results are shown in Figure(2 &3).

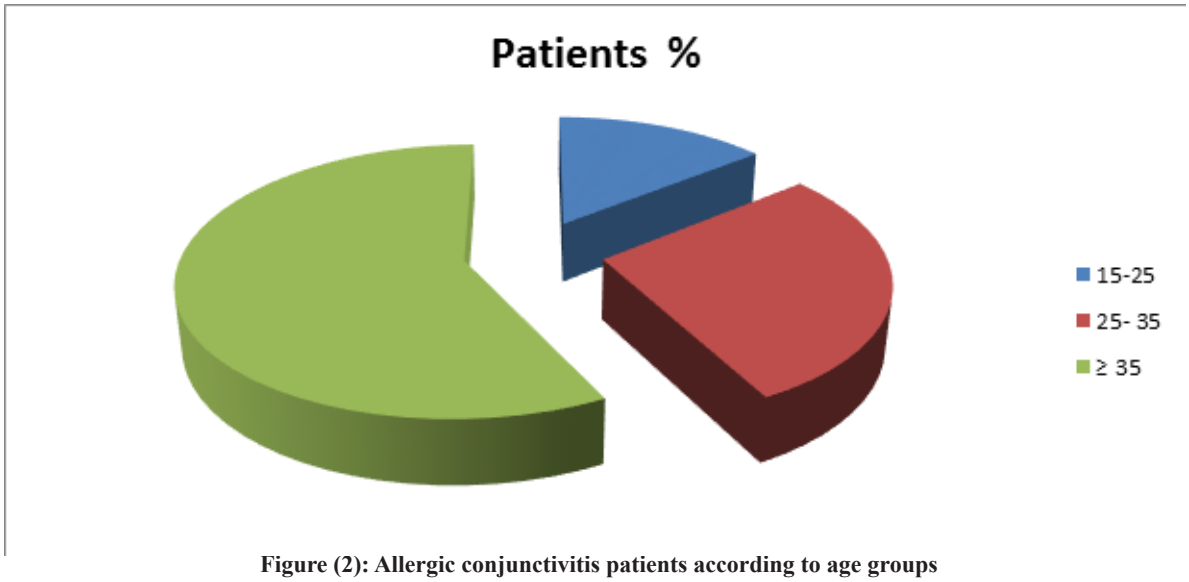

Figure (2): Allergic conjunctivitis patients according to age groups

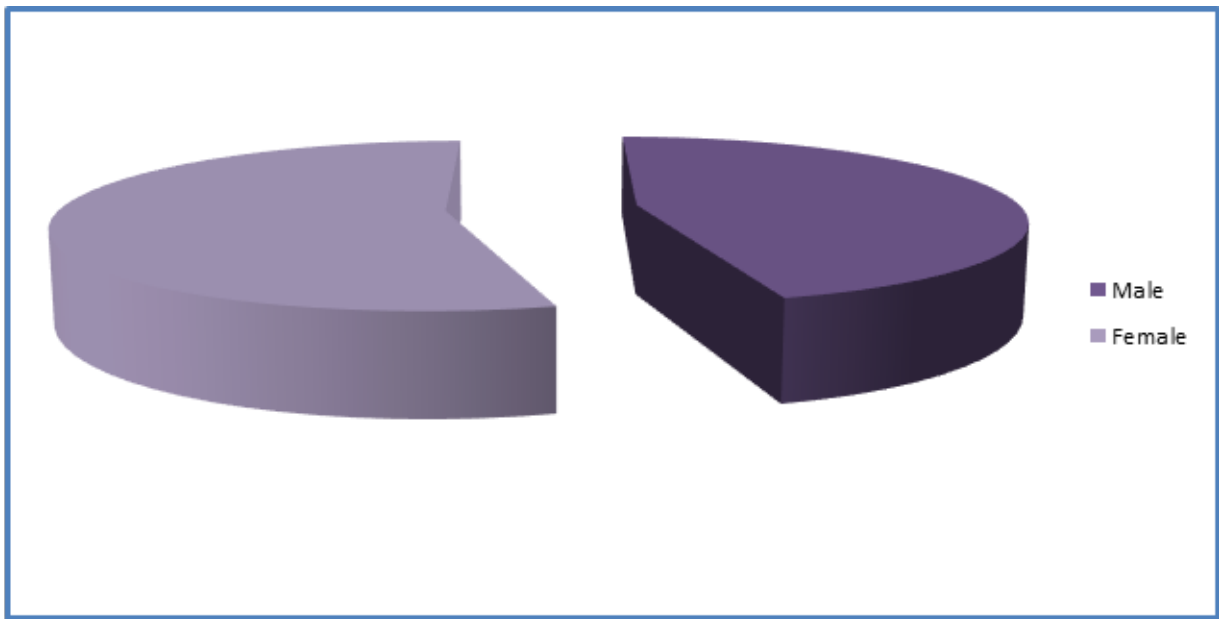

Figure (3): Allergic conjunctivitis patients according to gender

Table (2): comparative of interleukins Level (IL – 4 and IL – 10) in patients (Study group) and healthy (Control).

Levels of interleukins (pg/ ml)	Study group (M± S.D)	Control (M± S.D)	
IL - 4	23.91± 2.09	21.02± 2.99	No significant
IL - 10	14.99 ± 2.30	11.10± 1.99	significant

Results in table (2) showed the level of IL – 4 in allergic conjunctivitis patients was (23.91± 2.09) compare to control (21.02± 2.99) , so significantly increased levels of IL-10 in allergic conjunctivitis patients (14.99 ± 2.30) than control (11.10± 1.99),these results were shown in Fig.4.

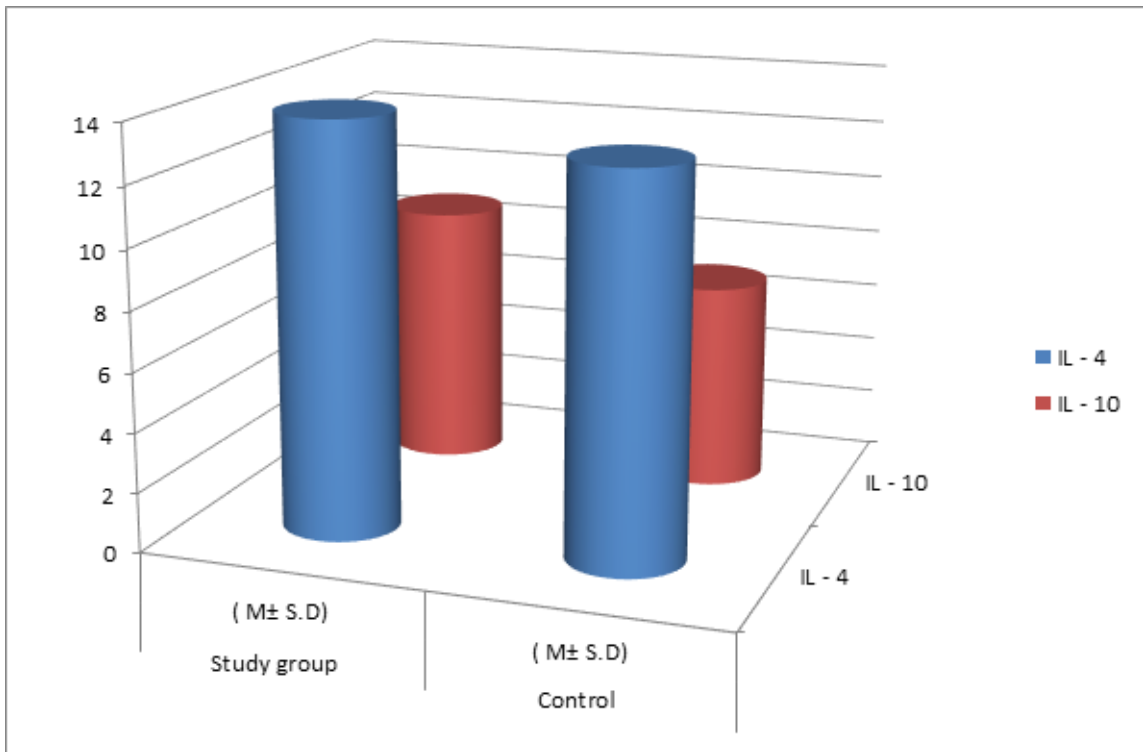

Figure (4): Comparative levels of interleukins (IL – 4 and 10) in allergic conjunctivitis patients and healthy.

Discussion

Current study showed more prevalence of allergic conjunctivitis patients in age group (≥ 35 years) as percentage (57.6%) compare to age group (25- 35) as (28.1%) , this similar results with Fasasi etal., 2014 who showed more prevalence of AC in age groups (17 -33) years as (43.6%), followed by ≥16 years as (42.3%)[8]. Ocular allergy is a higher prevalence in the western countries comparing to Africa and Asia[9]. Allergic conjunctivitis(AC) in (12–13) year old schoolchildren, was(19%)[10] , about (6–30) % of general population, who diagnoses with Allergic conjunctivitis[11], who represents ≥ 90% of all ocular allergies[12].

The current study showed the allergic conjunctivitis are commoner in females (54.5%) than males (45.5%). These results are consistent with the results of previous studies as[13;14;8] ,whilst differ with the results of researcher Hall and Shilio, 2005, who showed males more predominate than females[15].

Both Interleukin (4 and 10) are anti-inflammatory cytokines which mainly functions via suppressing the pro-inflammatory[16] .

During active the inflammatory of allergic eye diseases, the cytokines of T-helper cell (Th1 and Th2) were secretion and over expressed[17].cytokines of Th2 as interleukins (IL- 4; IL-5 ; IL- 9 ; IL -10 and IL-13) will promoting different elements of allergic inflammation as (isotype-switching from IgG1 ; propagation of the Th2 phenotype) to mobilization eosinophil ; maturation and activation mast cell and synthesis immunoglobulin (IgE), that important for allergy[18]

Interleukin (IL)-4 plays a very important role in inflammatory and fibrotic events in many human diseases[19], In Inflammation, such as post-cataract surgical or non-allergic cause slightly increased in production level of IL-4, but high produced level from IL-4 were related to allergic reactions[20], and increased level in the serum of allergic individuals[21], so IL-10 an anti-inflammatory cytokine (Th2-type cytokine) is secreting by different cell types as (B-cells ; T cells ;

macrophages and monocytes under various conditions of immune activation[22] .although IL-10 is been shown to suppress a broad range of inflammatory responses and is known to be an important factor in maintaining a balance of overall immune responses[23].Thus, used IL-10 in developed novel therapy for many human diseases such as autoimmune diseases and allergic responses[24] .

Conclusions

1. Significantly higher of allergic conjunctivitis patients in age group (≥ 35 years) , also significantly higher in females than males.

2. High level of IL – 4 in allergic conjunctivitis patients was (23.91± 2.09) compare to control ,so significantly increased levels of IL-10 in allergic conjunctivitis patients compare to control .

Conflict of Interest : None

Source of Funding : Self

Ethical Clearance : The sample taken after patient approval.

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The Role of Zinc Sulfate in Subchronic Cadmium Chloride Toxicosis in Male Mice

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Abstract

Aim: study sort of interaction between zinc sulfate and cadmium chloride. **Method:** sixty albino male mice divided equally into four groups as follows (Toxic dose of cadmium chloride 5mg/kg B.W (T1); Zinc sulfate 10mg/kg B.W (T2); their combined dosing (T3) and control group. (C) given D.W. At the end of the experiment, all the animal sacrified and their liver is dissected and prepared for histopathology also, another part of same liver submitted to homogenization process to determine the level of metallothionein 2 (MTII) and malondialdehyde (MDA). **Result:** histopathology of liver section revealed in the cadmium dosed group, a necrosis in hypatocyte, vascular degeneration, diffusion of mononuclear cell and neutrophill infiltration around dilated blood vessels, congestion of blood vessels around portal area and parenchyma, apoptosis of hepatocyte characterize by fragmented nuclei with irregular cytoplasm. While no lesion were recorded in liver section of zinc sulfate dosed group. In combined dosed group, liver section showed either no lesion or less severe lesion consisting of local aggregation of active macrophage and lymphocyte.Result of MTII in liver homogenate recorded highly significant increase in all treated group especially in combined dosed one that showed increase up to 3.3 times; while cadmium and zinc alone dosed group recorded increase 2.1 and 1.9 than control one. The result of MDA in liver homogenate showed highly significant increase level in cadmium toxic dosed group up to 17.8 times, while zinc sulfate group showed only 1.6 time increase than control and combined dosed group that showed no level differences. **Conclusion:** result of liver histopathology and MTII / MDA levels were indicative the protective role of zinc administration to overcome sub chronic cadmium toxicosis by increase their MTII binding protein that reduce the toxic effect or levels of cadmium induce cellular lipid peroxydation damage.

Key Ward: *MTII, MDA, Histopathology and liver homogenate.*

Introduction

Cadmium (Cd) is one of the most dangerous heavy metals, known to produce severe and multi-organ toxicity in humans [1,2]. It is released into the environment by mining, smelting operations, fuel combustion and other industrial process [3] Humans can be exposed to Cd and it can stimulate free radical production, resulting in oxidative deterioration of lipids, proteins, and DNA, manifesting as gross pathology of the liver, brain, and other organs in humans and animals [4]. The extremely long biological half life of cadmium essentially makes it a cumulative toxin, so long past exposures could still result in direct toxic effects of the residual metal [5] The body has limited capacity to respond to cadmium exposure, as the metal cannot undergo metabolic degradation to less toxic species and is only poorly excreted, making long-term storage a viable option for dealing with this toxic element. The long residence time of cadmium is in part attributable to metallothionein (MT), a metal-binding protein that is induced by cadmium and tightly binds the metal. Cadmium accumulates primarily in the liver and kidney where it is bound to MT, and it is felt that cadmium bound to MT is essentially detoxicated, at least temporarily, through this high affinity sequestration [6, 7]. Major detoxification mechanisms protecting the cell from Cd-induced damage is the direct binding of Cd2+ to metal chelators like Metallothionein (MT) is a ubiquitous low molecular weight (usually <10 kDa) protein with high cysteine content and has strong affinity for heavy metals [8].

The other way to induction (MT) is giving zinc which is an essential trace element play important roles in structure and function of proteins, metabolism of RNA and DNA, signal transduction, gene expression, and regulation of apoptosis [9]. It is required for the action of more than 200 metalloenzymes and more than 2000 Zn dependent transcription factors have been recognized [10]. Zinc has antioxidant properties and plays an important role in scavenging reactive oxygen species, and they also hypothesized that in the absence of zinc, the possibility of increased oxidative damage exists [11]. Several research showed that treatment with Zn during Cd exposure prevented or decreased the harmful effects of Cd [12]. Zn might reduce uptake of Cd by competing for a common transporter [13].

Moreover, zinc induced the synthesis of metallothionein in the liver, which caused Cd accumulation in the liver and delayed its transfer to the kidney. The zinc is a well-established antioxidants and it can protect against Cd-induced oxidative stress [14]

Method

In this study we used sixty adult albino mice weighted (30–32 g) that were divided into four groups of 15 for each group. They were administered the dosage daily orally at morning with overnight fasting by using gavages needle lasted for 45 days.

The first group of mice was (T1) administered cadmium chloride once a day at (5mg /kg B.W). The second group (T2) administered dose in the NOAEL range of zinc sulfate (10 mg/kg B.W) orally. The third group(T3) administered combination of both zinc sulfate (10 mg/kg B.W) and after four hours administered cadmium chloride(5mg /kg B.W) (1, 15). The forth group act as a control (C) and administered distilled water orally. At the end of sub chronic study all groups of animal sacrified surgically under anesthesia and their liver submitted to homogenization process to determine metallothionein 2 (MTII) and malon dialdehyde MDA level in liver, while another part of liver used for histopathological examination.

Findings

The table (1) showed a significant (P<0.01) increase in MTII level in liver homogenate of all treated groups after sub chronic dosing. The cadmium chloride group level increased by 2.17 times from the control group; while the zinc sulfate increase by 1.9 times that control one. The combined group showed the largest increase between all treated groups by triplicate level 3.3 times above the control level.

Table (1) Metallothionen II (MTII) level (ng/ml) in liver homogenate of mice groups dosed sub chronically with cadmium chloride and zinc sulfate and their combined:

Group n:15	MT2 In liver Mean ± SE
(T1)Cadmium Cloride 5mg/kg	8.17 ± 0.10 b
(T2)Zinc Sulfate 10mg/kg	7.32 ± 0.08 c
(T3)Cadmium and Zinc 5mg/kg+10mg/kg	12.74 ± 0.28a
(C)Control Distilled water	3.85 ± 0.12 d
LSD value	0.356 **
Means having different letters in same column differed significantly. ** (P<0.01).	

Table (2) listed that the level of MDA in liver homogenate showed higher significant increase (p>0.01) in cadmium chloride group with lowest increase but still significant in the zinc sulfate group while the MDA in liver seem to return to normal in the combined dosed group without significant difference from the control group.

Table (2) Malondialdehyde (MDA) level (nmol/g) in the liver of mice groups dosed sub chronically for 45 days with cadmium chloride , zinc sulfate and their combination :

Group n:15	MDA In liver Mean ± SE
(T1)Cadmium Chloride 5mg/kg	47.58 ± 0.19 a
(T2)Zinc Sulfate 10mg/kg	4.30 ± 0.18 b
(T3)Cadmium and Zinc 5mg/kg+10mg/kg	2.68 ± 0.24 c
(C)Control Distilled water	2.67 ± 0.19 c
LSD value	0.615 **
Means having different letters in same column differed significantly. ** (P<0.01).	

The result of histopathology showed in the control group normal liver section with no lesion in (fig.1 Section A)., while in cadmium group liver section showed infiltration of neutrophils and mononuclear cell around dilated and congested blood vessels (fig.1 Section B). There was a diffuse of mononuclear cells infiltration in liver parenchyma(fig.1 Section C) and in portal area(fig.1 Section D). A congested blood vessels and necrosis of hepatocytes seen in(fig.1 Section E).

Granulomatous inflammation were seen in the liver parenchyma in(fig.1 Section F). Also there were apoptotosis of hepatocytes characterized by dense nuclei chromatin with irregular cytoplasm in addition to fragmented nuclei (fig.1 Section G). Vacuolar degeneration and necrosis of hepatocytes also seen(fig.1 Section H). The zinc group showed normal liver section with no clear lesion at NOEAL dose (figure 9). The combined group showed normal liver cell with no clear lesion (fig.1 Section J)., while other tissue appeared with some granuloma consististing from local aggregation of active macrophages and lymphocytes (fig.1 Section D).

A- Section in the liver of control shows no clear lesions (H& E stain 40X)

B- Section in the liver of cadmium chloride group (T1) shows neutrophils and mononuclear infiltration around dilated blood vessels (H& E stain 40X)

C- Section in the liver of cadmium chloride group (T1)shows diffuse mononuclear infiltration in liver parenchyma (H& E stain 40X)

D- Section in the liver of cadmium chloride group (T1) shows diffuse mononuclear infiltration in portal area (H& E stain 40X)

E- Section in the liver of cadmium chloride group (T1) shows congested blood vessels with necrosis of hepatocytes (H& E stain 40X)

F- Section in the liver of cadmium chloride group (T1) shows granulomatous inflammation in the liver parenchyma (H& E stain 40X)

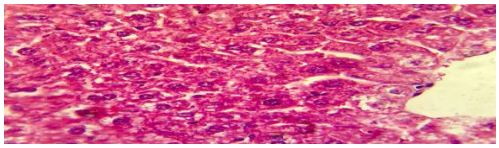
G- **Section in the liver of cadmium chloride group (T1)shows apoptotosis of hepatocytes characterized by dense nuclei chromatin of irregular cytoplasmic of hepatocytes in addition to fragments of nuclei (H& E stain 40X)**

H- Section in the liver of cadmium chloride group (T1) shows vacuolar degeneration and necrosis of hepatocytes (H * E stain 40X)

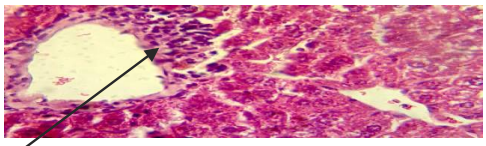
I- Section in the liver of the zinc sulfate group (T2) shows no clear lesions (H& E stain 40X)

J- Section in the liver of the combined dosing group (T3) shows no clear lesions (H& E stain 40X)

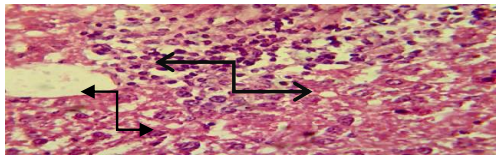
K- Section in the liver of combined group (T3) shows granuloma consisting from the local aggregation of active macrophages and lymphocytes (H& E stain 40X)



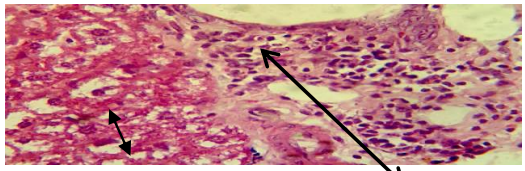
A- Section in the liver of control shows no clear lesions (H& E stain 40X)



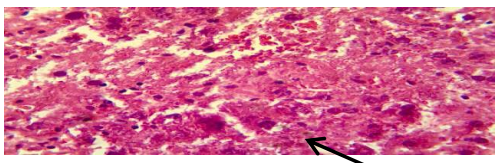
B- Section in the liver of cadmium chloride group (T1) shows neutrophils and mononuclear infiltration around dilated blood vessels (H& E stain 40X)

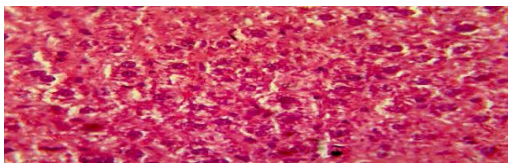


C- Section in the liver of cadmium chloride group (T1)shows diffuse mononuclear infiltration in liver parenchyma (H& E stain 40X)



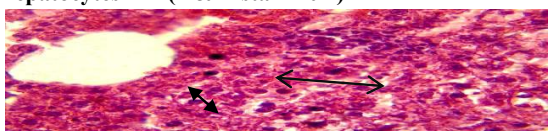
D- Section in the liver of cadmium chloride group (T1) shows diffuse mononuclear infiltration in portal area (H& E stain 40X)



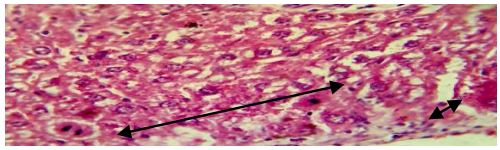


J- Section in the liver of the combined dosing group (T3) shows no clear lesions (H& E stain 40X)

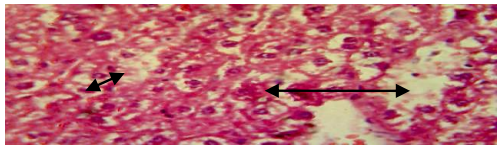
E- Section in the liver of cadmium chloride group (T1) shows congested blood vessels with necrosis of hepatocytes (H& E stain 40X)



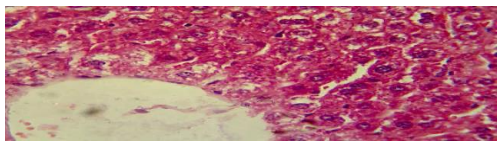
F- Section in the liver of cadmium chloride group (T1) shows granulomatous inflammation in the liver parenchyma (H& E stain 40X)



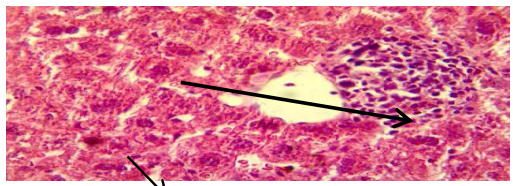
G- Section in the liver of cadmium chloride group (T1)shows apoptotosis of hepatocytes characterized by dense nuclei chromatin of irregular cytoplasmic of hepatocytes in addition to fragments of nuclei (H& E stain 40X)



H- Section in the liver of cadmium chloride group (T1) shows vacuolar degeneration and necrosis of hepatocytes (H * E stain 40X)



I- Section in the liver of the zinc sulfate group (T2) shows no clear lesions (H& E stain 40X)



K- Section in the liver of combined group (T3) shows granuloma consisting from the local aggregation of active macrophages and lymphocytes (H& E stain 40X)

Figure (1) Section in the liver

Discussion

The present study was designed and performed to study the interaction between zinc and cadmium and their combined dosing by performing dosing of them sub chronically for 45 days.

The hypothesis that Zn can protect tissue against Cd-induced tissue damage in adult mice and rats was established by our and several other previous studies [4, 16, and 17]. It is well known that many toxic effects of cadmium (Cd) action result from interactions with essential elements including zinc (Zn). These interactions can take place at different stages of absorption, distribution in the organism and excretion of both metals and at the stage of Zn biological functions. Exposure to Cd leads to disturbance in Zn level in the organism on the one hand, while dietary Zn intake has an important effect on Cd absorption, accumulation and toxicity on the other. The Zn status of the body is important in relation to development of Cd toxicity. Numerous data showed that increased Zn supply may reduce Cd absorption and accumulation and prevent or reduce the adverse actions of Cd, whereas Zn deficiency can intensify Cd accumulation and toxicity [18].

This is in accordance with the fact that the majority of Cd uptake occurs by a process associated with Zn transport, competing for common binding sites and membrane carriers like divalent metal transporter1 (DMT1) or luminal Zn transporter1 (ZTL1). Also, as oral treatment with Zn induces enhanced metallothionein (MT) synthesis in the liver [19].

Metallothionein is efficient intracellular small protein scavenger involved in intracellular detoxification via binding to cadmium. There was a significant increase of metallothionein concentration in all treated groups. The result of MT in sub chronic study indicate that MT level in liver homogenate increase by 2.12 times for Cd and two times for zinc alone group in comparison with control one but MT level in liver of the combined administered group showed higher increase of MT 3.3 times than control.

This could be attributed to the long time of exposure (45 day) that give opportunity for each metal to highly induce synthesis of MT in liver which possibly cause complete protection in Zn alone group while still in Cd a lone group which showed toxicity signs that increase with increase of administration period indicating that the induced MT were not enough to overcome the toxicity signs. And that in agreement with [19,20]. Binding and induction of metallothionein appears to play an important role in the physiologic regulation of zinc levels and, possibly, its reactivity to other legends [21].

The significant increase of the MDA level in sub chronic dosing in cadmium group might be due to increase of the oxidative stress and generation of the free radicals by cadmium. The cadmium group caused an increase of MDA level by 17.6 fold above control group while the combination group, the value of MDA returns to normal one. The reason of that was due to the role of zinc as antioxidant agent. It is well known that Zn is an essential component of the oxidant defense system with participation at multiple cellular levels and improved activity of antioxidant enzymes and as a result minimized oxidative damage [22].

Oxidative stress is considered an important mechanism of cadmium induced toxicity which might be due to the depletion and changes in the activity of antioxidant enzymes or reduced of glutathione (GSH) [23]. Another study recorded that the cadmium caused decreases in the activity and level of antioxidants system elements as well as vitamin C, E and glutathione content and leads to the production of oxygen reactive forms. Also by other route the cadmium toxicity generates free radicals by stimulating the synthesis of inflammatory mediators which in turn stimulates the generation and subsequent oxidative stress [24].

The liver is the primary target organ following systemic Cd exposure. The uptake of Cd into the liver is critical for the development of overall toxicity induced by heavy metal. Approximately half of Cd absorbed systemically is rapidly accumulated in the liver, which resulted in the reduced availability of Cd to such organs as the kidneys and testes, which are more sensitive to its toxic actions [25].

The hypatotoxicity involves two pathways, one for an initial injury produced from direct effects of the metal and the other for the subsequent injury produced by inflammation. Primary injury is produced by the binding of Cd to sulfhydryl groups on critical molecules in mitochondria, causing oxidative stress, the mitochondrial permeability transition, and decreased mitochondrial respiration.

Hepatocellular injury occurs because damage to endothelial cells disrupts the microcirculation and causes ischemia. Secondary injury from acute cadmium exposure is thought to occur from the activation kupffer cells and a complex series of interactive events with a large number of inflammatory and cytotoxic mediators[24].

The histopathological studies of the liver section in cadmium group showed mononuclear inflammatory cells infiltration of in the portal area, infiltration of neutrophils and mononuclear cell around dilated and congested blood vessels, congested blood vessels and necrosis of hepatocytes, which is a damage to membranes by severe, lysosomal enzymes enter the cytoplasm and digest the cell, and cellular contents leak out. That might happen due to oxidative stress and increase the tension on cells membrane lead to lake out of lysosomla content [26].

Histological alterations in liver tissues like degeneration of hepatocytes, vacuolization, congestion of hepatic tissues, subcapsular vacuolization, necrosis, were observed in the liver of mice exposed to Cd.

The initial lesion in the liver during the present study might be due to physiological changes that took place in the liver tissue in the process of trying to homeostatistically regulating and detoxifying the Cd metal during continuous exposure.

In hepatic tissue, the histological alterations noted during the sub chronic Cd exposure as focal necrosis, increased condense and fragment nucleus, cellular necrosis and ruptured hepatic tissue in Cd group. These findings are consistent with cadmium inducing greater hepatic alteration. Further, in this study identified alterations of the liver cells may be the result of diverse biochemical alteration in liver following the Cd toxicity that act as a signal of degenerative processes that suggests metabolic damage also [27].

In addition to above chances vacuolation of hepatocytes is also noted which is suggested to be associated with the inhibition of protein synthesis, energy depletion or a shift in Cd as a poisonous metal that promotes early oxidative stress and later contributes to the development of serious biochemical and pathological conditions because of its long retention in some tissues.

In the present work, the administration of Cd resulted in severe hepatocyte necrosis, fatty changes, degeneration signs and inflammatory cell infiltrations. These results are similar to those reported previously in the literature [28].

Conclusion

Zinc sulfate at low dose might act as antidote in case of cadmium toxicosis this was supported by the result of clinical physiological as well as the level of zinc and cadmium in liver.

Conflict of Interest: There was no conflict with this paper

Funding: Self

Ethical Clearness: This work done under the rule of ethics for management laboratory animals submitted by university of Baghdad - college of veterinary medicine.

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Correlation Between Glugometer and Laboratory Methods in Measuring Capillary and Venous Blood Glucose Levels in Type 2 Diabetic Patients

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Abstract

Objectives: The aim of the study was to investigate the correlation between levels of blood glucose in samples taken from patients with type 2 diabetes mellitus measured by instrument for self-monitoring of blood glucose (glucometer) and by laboratory methods (photometric method or enzymatic colorimetric method). **Method:** One hundred patients with type 2 diabetes mellitus (T2DM) who were attending a privet medical laboratory during the period December 2017 to March 2018 were participated in the present study. Among the participants, 46 were female with an age range of 40-80 years and a age mean of 53.10± 10.61 years .The male patients were 54 with an age range of 40-79 years and a age mean of 51.17± 9.74 years. Venous and capillary blood glucose levels were determined by laboratory methods and glucometer for each participant. **Finding :** The mean of photometric venous blood glucose (PVBG) was 163.88 ±107.06, Glucometric Venous Blood Glucose (GVBG) was 190.95 ±123.96 mg/dl, Photometric Capillary Blood Glucose (PCBG) was 171.53±116.52 mg/dl, and Glucometric Capillary Blood Glucose (GCBG) was 193.69±123.21 mg/dl. The obtained data was analyzed by Lin's concordance correlation coefficient. Concordance correlation coefficients (r_c) were 0.94 for PVBG vs GVBG and 0.97 for PCBG vs GCBG.***Conclusion:***

The results obtained in this study showed that the values of blood glucose level determined by glucometer are close to the values determined by laboratory methods. Moreover, a positive correlation coefficient showed strong association between capillary and venous glucose measurements.

Keywords: *glugometer; laboratory methods ; capillary ; venous; blood glucose ; type 2 diabetic patients*

Introduction

In a global report on diabetes, WHO has noticed an increase in both the prevalence of diabetes and the number of diabetic people. The report has emphasized the need of sufficient lifelong management and regular follow-up for diabetic patients so they can have healthier lives [1].

It is well known that diabetes is associated with complications such as blindness, cardiovascular disorders, limb amputation and nephropathy [2].

Reaching a reasonable glucose control in patients with diabetes depends on the availability of blood glucose (BG) level knowledge. To get this knowledge, patients may check their BG level at hospitals or privet medical laboratories where biochemical methods are applied to measure BG levels [3].

Regular self-monitoring of blood glucose (SMBG) by diabetic patients was recommended by clinical guidelines as a tool for self-management [4]. Self-monitoring of blood glucose has been considered as a fundamental part of self – management and diabetes care as it has many benefits .These benefits include the use of SMBG for the detection of serious hyperglycemia and as an advance notice to detect hypoglycemia. Moreover, real-time data can indicate the effects of life style, such as diet and physical activity, on blood glucose levels[5]

Rapid blood glucose determination has become a requirement for treatments and dose adjustments. Laboratory methods to measure plasma glucose levels are time consuming. Therefore, the use of glucometers has greatly increased [6].

Using glucometer device in SMBG makes it a process where Blood Glucose (BG) is checked by the patient himself. As an out-come of using glucometers, the patients are participating in the therapy process and hence they would have a better life quality [2]

Beside better glycemic management, the benefits of using glucometers include simple usage and operation and the need of one drop of blood. These benefits are not reduced by doubts regarding the precision and accuracy of the devices [2].

According to the international organization for standardization (ISO) acceptable error for a glucometer be within ± 15 mg/dl when laboratory glucose levels are <100 mg/dl; and the acceptable error should be within 15% for laboratory values ≥100mg/dl[6]

The present study was conducted to investigate the correlation between blood glucose concentrations determined by a glucometer and blood glucose levels obtained by laboratory method.

Materials and Method

Measurements were based on two types of blood samplings, venous blood sampling and finger-prick blood sampling. Blood glucose levels of samples collected by the later method of sampling were determined by glucometer and capillary laboratory method.

For the laboratory (photometric) blood glucose level measurements, a 3.5 ml of venous blood samples were collected from participants by applying the blood drawing at the median cubital vein, whenever possible, following normal protocol. A small portion of each sample was used to measure glucose concentration with glucometer. Sodium fluoride was used as glycolysis inhibitor. Blood samples were kept in an appropriate conditions and environments for subsequent analysis.

Capillary sampling from a finger was performed for each and every participant. The entry site was disinfected; the skin was punctured with a lancet. The first drop of blood was wiped away then a blood sample was collected with a capillary tube. A micro hematocrit centrifuge was used at 12000 rpm to separate serum.

The blood glucose levels were determined using an enzymatic colorimetric method according to the kit's protocol using a spectrophotometer .The kit type Linear (LINEAR CHEMICALS, SPAIN) was purchased from local markets.

Glucometric measurements of blood glucose level were performed using a Glucometer (ACCU-CHEK Active Mannheim, Germany). Glucometric measurements were applied for capillary blood glucose (GCBG) and for venous blood samples (GVBG).

Photometric measurements of blood glucose level were performed using a Uv/Visible Spectrophotometer (Type Apel – china). Photometric measurements were applied for capillary blood glucose (PCBG) and for venous blood samples (PVBG).

Finding

One hundred diabetic patients were participated in this study. In table 1 the statistical data of the participant are presented.

Table 1-Statistical data of the participant patients

	Female	**Male**	**Total**
Sample size	46	54	100
Age range, Years	40-80	40-79	40-80
Mean ± s.d*., years	53.10 ± 10.61	51.17 ± 9.74	± 10.16

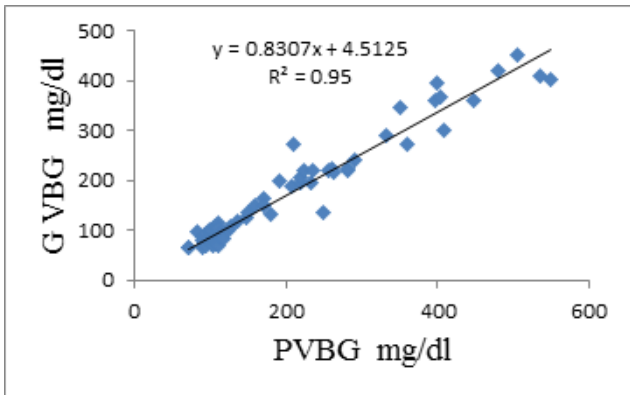
*standard deviation

The experimentally obtained blood glucose levels that were measured by glucometric and photometric techniques are summarized in table 2. The mean value of venous blood glucose level and capillary blood glucose level along side with standard deviation are given in table 2.

Table 2. Comparison of different glucose level determination methods. methods and the corresponding correlation coefficients

	Venous blood		Capillary blood	
	Glucometer	**Photometric**	**Glucometer**	**Photometric**
Mean Glucose level ± s.d. mg/dl	190.95 ±123.96	163.88 ±107.06	193.69 ± 123.21	171.53 ±116.52
Correlation coefficient ,R^2	GVBG vs PVBG		GCBG vs PCBG	
	0.95		0.992	
Concordance correlation coefficient, r_c	0.94		0.97	

To assess the strength of association between the glucose levels obtained by the two measurements methods, the linear correlation coefficient R^2 were determined from the linear plots of GVBG vs PVBG and GCBG vs PCBG which are shown in figure 1-a and figure 1-b respectively.



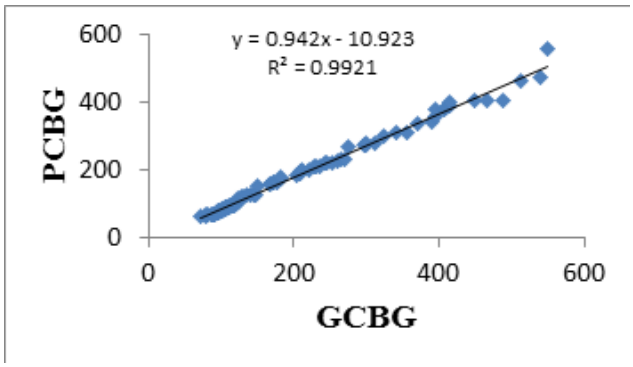


Figure 1- linear correlation plots of : a –PVBG vs GVBG and . b- PCBG vs GCBG

The values of the square of the correlation coefficient, R^2 are given in table 2 These values indicate a high association between glucometric and photometric methods used for blood glucose level measurements. The case of PCBG vs GCBG showed higher association, as the corresponding R^2 has a value of 0.992, in compression to PVBG vs GVBG case where R^2 has a value of 0.95.

The obtained data were further analyzed by the procedure which calculates Lin's Concordance correlation coefficient [7]. The procedure assumes that n observations Y_k and X_k are selected from a bivariate population. Here Y represents a measure from a candidate method and X represents the corresponding measure of the standard test or method, in the present study it is the photometric method. The degree of concordance (agreement) between the two measures is represented by the value of r_c which can be estimated from the formula :

$$r_c = \frac{2S_{XY}}{(Y-X)^2 + S^2_Y + S^2_X}$$

The statistic, r_c , is an index of how well a new test or measurement (Y) reproduces a standard test or measurement (X). It quantifies the agreement between these two measures of the same variable. The values of a correlation, r_c ranges from -1 to 1, with perfect positive agreement at 1[7].

Concordance correlation coefficient (r_c) analyses values are presented in table-2. Those values showed moderate agreement between GVBG and PVBG with r_c value of 0.94. A strong agreement was observed for the correlation between GCBG and PCBG as the corresponding r_c values was 0.97.

Conclusion

The results obtained in this study showed that the level of blood glucose determined by glucometer is close to the blood glucose level measured by laboratory methods. Moreover, a positive correlation coefficient showed strong association between glucometric and photometric methods.

The concordance correlation coefficient (r_c) showed a high agreement between laboratory methods and the use of glucometer.

Hence, from the obtained results of the current study it may be concluded that use of glucometer is benefiter for glucose self monitoring to asses the control of blood glucose level for diabetic patients.

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Conflict of Interest : Non conflict of interest with any side

Source of Funding : Self source

Ethical Clearance: Nil

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Effect of *Toxoplasma gondii* Infection on DNA Sequence among patients with Testicular Cancer

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Abstract

This study was conducted on 20 testicular cancer patients who visited the Al-Barraa privet Laboratory and Al- Sharika Labratory / Baghdad, with mean age of (27.77 ± 6.01) years. The ELISA technique was used to detect toxoplasmosis, and tumor biopsies were taken from 6 patients. The PCR amplifications of the "PIK3CA" gene were performed. The primers were used for exon 9 amplification. During the exon 9 PIK3CA gene amplification process, the first pair of the used primers included PIK3CA-9F-1, "5′- GTATTTGCTTTTTCTGTAAATCATCTG-3" and "PIK3CA-9R-1, 5′ CATGCTGAGATCAGCCAAATTC-3′′. The determination of PIK3CA genome was done by nested polymerase Chain Reaction using primers of PIK3CA gene with amplicon size 161bp. The "oligonucleotide" was tested at 0.05, 0.075 and 0.10 µmol/L concentrations, while ""LNA oligonucleotide" was tested at 0.075 and 0.0375 µmol/L concentrations for *PIK3CA* exon 9", which showed optimal detection for the *PIK3CA* exon 9 LNA mutation.

Key word: *Toxoplasma gondii, Testicular Cancer, DNA sequence.*

Introduction

Toxoplasmosis is a serious condition in which individuals are infected and may then suffer from cancer [1,2]. Toxoplasmosis can reach to most organs of the human body and the latest genetic change in some of these organs may result in carcinoma [3].Testicular cancer is the heterogeneous disorder which was historically shown to be challenge for classification [4]. High levels of their heterogeneity is explained by the emergence of pluripotent germ cell lines, and by long time interval when the oncogenic mutations assemble prior to the quickly invasion growth during or post puberty stage [5]. "TGCT originates from the aberrant arrested fetal gonocyte", which does not progress into "spermatogonium appropriately after birth". During puberty and childhood, this arrested gonocyte shows oncogenic adaptation, which then become germ cell neoplasia in situ (GCNIS) during childhood and adulthood, which then emerges as an invasive TGCT during young adulthood. During the early childhood, GCNIS can be identified histologically, but during the young childhood, it is challenging and difficult to be differentiated from the normal germ cells [6]. In general, histologic classification shows two subtypes of "TGCT", the "Seminoma and "Non-Seminoma" TGCTs. The seminoma is a homogenous cancer similar to the undifferentiated gonocytes, and accounts for about (55%) of the TGCTs, and its peak incidence occurs at the age group (35-39) year, while a "non-seminomatous germ cell tumor "(NSGCT)" constitutes about "(44%)" of the "TGCTs" and in general, they are more aggressive than seminomas, and diagnosed at younger ages "(25-29)" years. The heterogeneity of "NSGCT" composition reflects its dysregulation in differentiation into embryonic cancers, "choriocarcinomas, teratatomas and yolk sac tumors" [7]. The combined or mixed tumors are the tumors which contain both NSGCT and seminoma and classified as a NSGCT subtype. TGCT is usually defined as the curable model of cancer, which is has an exquisite susceptibility to chemotherapy, and has more than (95%) survival rate. Chemotherapeutical treatment of TGCT is usually associated with the morbidity and complications such as infertility, metabolic syndromes or cardiovascular diseases [8].

Materials and Method

In the current study, 20 testicular cancer patients were enrolled. The tumor biopsy specimens were taken from 6 patients, and the other 14 patients were analyzed

for *"PIK3CA"* mutations using PCR. *Elisa technique was used for detection of toxoplasmosis*. In the exon "9 PIK3CA" gene, the "prescreening for mutation detection was done by using direct DNA sequencing", as previously described. Further analysis was conducted on specimens with mobility shifts by the direct DNA sequencing on automated sequencing systems from ("ABI PRISMTM 3100 Genetic Analyzer, Applied Biosystems, Hitachi, Japan") using the "ABI PRISM Big Dye" Terminator version 1.1 Cycle Sequencing Ready Reaction Kit from (Applied Biosystems, Branchburg, NJ, USA).

Statistical analysis:

The Microsoft Office Excel 2007 and SPSS version 16 programs were used for data analysis. The numeric data were expressed as mean ± SEM (standard error of means). The P value (< 0.05) was considered as significant.

Results

Table (1) shows the distribution of testicular cancer according the age groups and residency of patients.

Table (1): Distribution of patients according to age groups and residency

Age groups	No=20))	100 %
(45-49)	5	25.0
(50-59)	5	25.0
(60-69)	6	30.0
(70-75)	4	20.0
Residency		
Rural	12	60.0
Urban	8	40.0

Table (2) "shows the mean toxoplasma (IgG) and (IgM) antibodies detected by ELISA technique".

Table (2): Mean toxoplasma (IgG) and (IgM) antibodies detected by ELISA technique

Disease	Mean IgM	Mean IgG
Toxoplasmosis	(0.48 ± 0.23)	(0.38± 0.20)

During the "exon" "9 PIK3CA" gene" amplification process, the first pair of the used primers included "PIK3CA-9F-1", "5′-GTATTTGCTTTT-TCTGTAAATCATCTG"-3′ and "PIK3CA-9R-1, 5′ CATGCTGAGATCAGCCAAATTC-3′" as shown in figure (1).

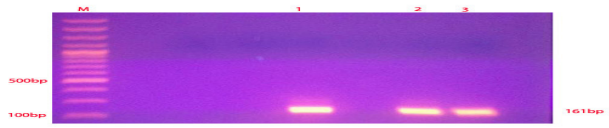

Figure (1): Determine of PIK3CA genome by nested polymerase Chain Reaction using primers of PIK3CA gene.

M: 100 bp DNA ladder (M). Lanes: (1-8) positive isolates (161 bp). At 2.5% agarose gel.

The Electrical current is equal to 60 volte at 30minutes.

Analysis of *PIK3CA* gene

The "oligonucleotid"e was tested at "0.05, 0.075" and "0.10 µmol/L" concentrations, while "LNA oligonucleotide" was tested at "0.075" and "0.0375" µmol/L concentrations for "*PIK3CA* exon 9", which showed optimal detection for mutation as seen in table (3).

Table (3): Polymerase Chain Reaction of *PIK3CA* Exon 9

"PIK3C4 Exon"	"Mutation"	"Nucleic acid"	"Sensitivity with" "LNA probe"
9	"E545K"	"GAC > AAG"	1.0%
	"E545D"	"GAG > GAT"	2.5%

Discussion

Testicular cancer is one of the most common condition observed in the elderly age. Involvement of *Toxoplasmosis* with this testicular histological change is controversial. Our study revealed that the (45-75) years age group is more infected with this disease. Epidemiology is one of the common conditions in Iraq and people suffering from this disease may suffer from other physical complications. This finding agreed with Jenna E. Boyd who found that there are many complications that may take place following the treatment of this malignant tumor [9].

Toxoplasmosis is an age-problem disease that affects most body parts causing apparent damages, and can be very serious causing malignant tumors [3]. Several body organs develop multiple impairments due to the migration of toxoplasma tachyzoites [10]. Thus, cancerous tumors can affect different parts in the body because of toxoplasmosis [11]. Several genetic mutations may take place due to the relationship between toxoplasmosis and various body organs and tissues accompanied by tissue alteration, which matches with the report of Ramakrishnan, Ch. *et al,* 2019, who demonstrated that *Toxoplasma* happens at the site of occupation to the body as a result of its interaction with the body tissues [12]. In our study, the genetic mutations were found in the gene E545K in Nucleic acid GAC > AAG and E545D gene in Nucleic acid GAG > GAT, in PIK3C4 *Exon* 9, where this genetic mutation occurred in this site of the genetic sequence of the DNA of the testicular tissue. Existence of *Toxoplasma gondii* at the affected site reinforced the genetic mutation in the testicular tissue. These findings agreed with Jun Zheng et al, 2019 who showed that A TCTP-like gene was detected in the genome of Toxoplasma [Toxoplasma gondiiTCTP (TgTCTP)], despite its unknown unknown function [13]. This means that the alteration in the epidermal tissue's genetic sequence is due to the existence of toxic toxoplasmosis precisely at the injury site, which can be attributed to the weak immune system of the infected individual, and thus leading to that malignant tumor.

Ethical Clearance: Taken from patients

Source of Funding : Self

Conflict of Interest : Non

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Correlated level of interleukin (IL_10) with Allergic Rhinitis and Effect Study of Steroid in its Levels

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Abstract

Background: the allergic rhinitis or hay fever, is the most common diseases or type of allergic, often associated with runny nose ; nasal congestion; sneezing ; sinus pressure and eye problems, that similar to cold, so in recent years allergic rhinitis increased in Iraqi population, because many important factors. The aims of the current study were to assessment (IL_10) in Patients serum and study the effect of Steroid in Its Levels.**Patients and method**: Patients' serums were collected form Salah Aldin General Hospital during period (November ,2018 - March, 2019) a total of 44 patients Suffering from Allergic rhinitis after diagnosis by physician ENT Specialist and control group included 32 (healthy person), the serum IL_10 levels that measured by theELISA (Beckmancompany, USA) according to the manufacturer's protocols (IL_10 ELISA kits), All samples are detected in duplicate. **Findings:** Mean of age ± Standard division was (29.17±7.65) compare to control 30.23±8.90 , high incidence allergic rhinitis in males 29(65.9%) than females 15(34.1%) , also in control group ;males18 (56.3 %)more than females14 (43.7 %); P<0.01. Also Results in of current study showed significant decreased levels of interleukin(10) in serum of Patients with allergic rhinitis as (8.21 ± 0.25), compare to control group (healthy persons) as(11.67 ± 0.33) differed significantly (P<0.01), but significant increased level of IL_10 (10.06 ± 0.23) in serum of Patients with allergic rhinitis who received gluococorticoid (steroid) Patients compare to Patients before received gluococorticoid (steroid), a differed significantly (P<0.01). **Conclusion:** high incidence Allergic Rhinitis in males than females. decreased level of (IL_10) in serum with allergic rhinitis but increased levels of IL -10 in Patients serum with allergic rhinitis who received gluococorticoid (steroid) Patients compare to Patients before received gluococorticoid (steroid).

Key words: *Patients ; Allergic Rhinitis ; Steroid ; IL10 Levels*

Introduction

The allergic rhinitis (AR), is a common disorder which is linked as strongly to conjunctivitis and asthma, nearly 500 million people in worldwide suffering from AR[(1)], so AR is I.g.E-mediated chronic inflammatory, which is considered a classic Th2-mediated disease[(2)]. the symptoms of allergic rhinitis asnasal obstruction ; nasal itching;sneezing as well as rhinorrhea.

Also the nasal and ocular symptoms directly related to the allergic process, as well as leads to sleepiness during the day and less the quality of live because of symptoms[(3)] .

Allergic rhinitis to development requires an interaction between: immune system ;environment factor as well asgenetic susceptibility. the Allergen inducing proliferation of Th-2 lymphocyte in patients with allergies with releasing of their characteristic combination of cytokines including interleukins IL(3; 4; 5 ; 9; and 13). These substances promoting producing the I.g.E and mast cell. Mucosal mast cells which producing the IL (4 ; 5 and 6) and tryptase proliferate in the allergic epithelium[(4)]

The importance of the Th2-type cells, (Th2) cytokines in both pathologies of allergic inflammation and the development the allergic sensitization and well established[(5)].The inflammatory response in the nasal mucosa in patients with allergic rhinitis, that challenging intranasally with the allergen including"immediate I.g.E-mediated mast cell response" and late-phase

response characterized by recruitment of basophiles; eosinophils[6].

Corticosteroids, one important type of steroid hormones, which taken as topical creams; nasal sprays ; pills as well as long-lasting injections. Glucocorticosteroids exert anti-inflammatory effects via at two pathways: transrepression pathway and transactivation pathway, so glucocorticosteroids exert regulatory functions by inducing regulatory cytokines and for khead box P3 (FoxP3) regulatory T-lymphocyte[7,8] .

Patients and methods:

Sample collection: Patients' serums were collected form Salah Aldin General Hospital during period (November ,2018- March, 2019) a total of 44 patients Suffering from Allergic rhinitis (A.R), with average age (29)years after diagnosis by physician ENT Specialist and control group included 32 healthy person who don't suffering from any infections(healthy persons) with average age (30) years.

Detection level of interleukin -10: levels of (L-10) were measuring by the ELISA (Beckman_USA) according to manufacturer's protocols (IL_10 ELISA kits), by pre-coated microtiter plate with an antibody specific to IL_10 , after then added the samples and standard to appropriate wells, then added to each wells in microtiter plate the biotin conjugated antibody and avidin conjugated to Horseradish peroxidase(HRP) and incubation, then adding TMBsubstrate solution for wells. terminated enzyme substrate reaction by adding sulphuric acid solution and by spectrophotometrically measure the colour change at a wavelength (450 nm). concentration of Interleukin-10 in serum of patients determine by compare O.D of samples with standard curve. All samples are detected in duplicate.

Data Analysis

The Statistical Analysis System- SAS (2012) [9] program was used to effect of difference groups in serum IL_10 in allergic rhinitis patients and effect of steroid in its levels. y.

Findings

Table 1: Demographic of the study groups (patients) and control group (healthy).

Variable	Patients	Control	p.value
Age (M ±S.D)year	29.17±7.65	30.23±8.90	P<0.01
Gender			
Males	29 (65.9 %)	18 (56.3 %)	P<0.01
Females	15 (34.1 %)	14 (43.7 %)	
Total	44 (100 %)	32 (100 %)	

Table (1) showed the mean of age ± Standard division was (29.17±7.65) compare to control (30.23±8.90), as well as results in this table showed high incidence allergic rhinitis in males 29(65.9%) than females 15(34.1%) ,also in control group the males18 (56.3 %) and the females14 (43.7 %) ; P<0.01.

Table 2. levels of IL_10 in serum of patients with control group .

Study groups	Level of IL_10 Mean of concentration ± SE (pg/ml)
patients with allergic rhinitis	8.21 ± 0.25
Control (healthy)	11.67 ± 0.33
P-value	0.0001
** (P<0.01)	

Results in table (2) showed significant level decreased of IL(10) in serum patients who have allergic rhinitis as (8.21 ± 0.25) , compare to control group (healthy persons) as(11.67 ± 0.33) differed significantly (P<0.01) (fig.1).

Table 3. Levels of (IL_10) in serum of patients with allergic rhinitis before and after received steroid

Study groups	Level of IL_10 Mean of concentration ± SE (pg/ml)
Patients before received steroid	8.21 ± 0.25
Patients after received steroid	10.06 ± 0.23
LSD value	0.761 **
P-value 0.0001	
** (P<0.01)	

Results in table (3) showed significant increased level of IL_10 (10.06 ± 0.23) in serum of Patients with allergic rhinitis who received gluococorticoid (steroid) Patients compare to Patients before received gluococorticoid (steroid), a differed significantly (P<0.01) (fig.1).

Discussion

Rhinitis is a common disorder is broadly defined as inflammation nasal mucosa. Current study showed high incidence Allergic Rhinitis in males than females,It affects more than 40% of the population of world[10]. The high incidence of new-onset AR was the youngest age group (20-29) years[11] .

Results in of current study showed significant decreased levels of IL_10 in patients serum with allergic rhinitis as (8.21 ± 0.25) , compare to control group (healthy persons) as(11.67 ± 0.33) differed significantly (P<0.01), but significant increased level of IL_10 (10.06 ± 0.23) in serum Patients who received gluococorticoid (steroid) Patients compare to Patients before received gluococorticoid (steroid), a differed significantly (P<0.01).

Whilst other studies, showed no significant difference was detected in Patients with allergic rhinitis and the control[12] .The efficacy of immune therapy for seasonal allergy rhinitis is confirmed but still not in perennial allergy rhinitis in UK, Immune therapy indicated: Evidence of I.g.E mediated disease ; Inability to avoid allergies ; Failure of drug therapy ; Compliance[13].IL10 decrease in Allergic rhinitis so there's reverse relation between IL10and I.g.E. IL10inhibits IL_2 and IL_5 production by $TH_1$and TH_2 , so it act as immune suppresser, and the IL10increase in patient received Glucocorticoid therapy (both act as immune suppressor), short course of oral prednisolone (20 mg) for (5) days are very effective for seasonal symptoms[13], administration of IL10 during allergic attack significantly reduced eosinophils and Mast cells as well as type2 cell helper that leading to decrease the inflammatory process, so it will reduced allergic rhinitis[13], as well as many previous studies showed significant increase in IL10 after immunotherapy [14]

The sodium cromoglycate or Cromolyn have the ability for educing sneezing; rhinorrhea and nasal itching , therefore a reasonable therapeutic option for patients, also anti-I.g.E antibody omalizumab has effective for asthma and seasonal allergic rhinitis[10]. So many studies appearance the relationship between cytokines with AR as IL-4 and IL-13[15].Corticosteroids are worked by decrease inflammation and suppress immune response[16] .

As well as therapy by glucocorticoid caused inducing the T-lymphocytes apoptosis in peripheral lymphoid organs and down-regulated adhesion molecules, and causing reduce the migrated T cells to the site of inflammation[17].

Conclusion

High incidence Allergic Rhinitis in males than females.

Decreased level of (IL_10) in serum of AR patients as but significant increased level of (IL_10) in serum of AR patients who received gluococorticoid (steroid) Patients compare to Patients before received gluococorticoid (steroid).

Recommendation :future study about consider IL_10 as a part of immunotherapy for patients with allergic rhinitis.

Ethical Clearance: from research ethic committee in Tikrit university/college of medicine

Source of Funding : Self

Conflict of Interest : None

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Association of Hyperuricemia with Knee Osteoarthritis and Generalized Osteoarthritis in Iraqi Patients

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Abstract

A number of 288 osteoarthritis patients involved in this study, their ages, sex, BMI .serum uric acid levels are recorded, knee joints x ray done for them, and classified into 5 grades (grade 0-grade4).serum uric acid level classified into 3 classes (low2.5.normal 2.5-6, high more than 6 mg\dl) establishing the diagnosis of knee degenerative arthritis according to criteria of ACR (American college of rheumatology. The aim of the stud was to find relation between serum uric acid level with knee osteoarthritis and generalized osteoarthritis in Iraqi patients . in this cross-sectional study, 288 patients involved their ages ranged from 45-90 years old mean age 67.5 years; female patients were 216 (75%). Mean age of females 62.5 years. Male patients 72 (25%). Mean age of males 67.5 years. The study shows highest rate of knee OA patients and generalized OA (76.21%,63.16 respectively) have normal level of uric acid ,while only low rate of knee OA patients and generalized OA(21.93%,31.58 respectively)have high level of uric acid which was NS (P=0.547) This cross sectional study conclude that there was no association between hyperurecemia and knee osteoarthritis nor generalized osteoarthritis

Key words: *hyperurecemia, knee OA, Generalized OA.*

Introduction

The risk of mobility and disability attributable to osteoarthritis (OA) alone is greater than any other medical condition among elderly [(1)]. The prevalence of OA that are symptomatic varies between 7 and 26% dependent on the site and definition of OA [(2)]. The burden of OA has been increasing in the past two decades worldwide and its prevalence is projected to be nearly double in the next decade [(3)]. Current treatment paradigms are limited to palliative measures broadly focused on analgesia and joint replacement for end stage disease. Gout is a crystal-induced arthritis caused by deposition of the monosodium uric acid crystal (MSU) related to long standing hyperuricemia. It is also a common inflammatory arthritis affecting around 5% of the middle-aged and elderly population worldwide [(1)]. The association of uric acid and OA has long been observed, and a pathological link between gout and OA has been hypothesized [(4)]. Aging and obesity are significant risk factors shared by both OA and gout, and may confound the association between both conditions[(5-7)]. Degenerative arthritis is the most well known joint arthritis worldwide[(1)]. The link with age, weight, gender and metabolic parts has been studied. [(3)]. These investigations demonstrated a relationship of degenerative arthritis with increase in body weight. A portion of these examinations represented serum uric acid and found no relationship among it and OA [(5)]. Davis *et al* explored a significant relationship among knee degenerative arthritis and uric acid yet a little one not achieving noteworthy level[(2)]. Sun Ye *et al* particularly explored serum uric acid association with osteoarthritis and presumed that albeit uric acid was related with generalized degenerative arthritis in patients experiencing hip substitution, there have been no relationship with knee degenerative arthritis or respective hip or knee degenerative arthritis[(8)]. Others revealed a relationship with degenerative of different joints[(9)]. While a study done in Saudi Arabia found a conceivable relationship among hyperurecemia and knee and generalized OA[(11)]. Suad A *et al* found a relationship between uric acid dimension and knee OA[(12)]. The aim of he current study was to explore the connection among serum uric acid and knee and generalized degenerative arthritis in Iraqi population.

Methodology

The present investigation included 288 patients seeing by rheumatologists in two noteworthy healing centers (Azadi Teaching Hospital and Kirkuk General Hospital) in Kirkuk city in the north of Iraq. Their age, sex, weight index (body mass index) (weight in kg separated by the square of length in meters), serum uric acid were recorded. Body mass index grouped by World health organization into following classes: Table 1.

Establishing a patient suffering from knee OA were according to 'The American College of Rheumatology criteria for diagnosis of rheumatic diseases' and these are:

- Knee pain.
- Osteophytes on X-ray.
- Crepitus on knee range of motion.
- Morning stiffness of short duration (<30 min).

Inclusion Criteria

Patients within the age group of 45-90 years of both sexes, who were suffering from primary Knee OA, were included in the study.

Exclusion Criteria

Patients aged less than 41 years and more than 90 years,

Pregnant women and those suffering from secondary OA,

Rheumatoid arthritis, Diabetes Mellitus, Renal insufficiency, Hepatic disease, gout or with any other systematic disease were excluded from the study.

Blood samples along with the radiographs of the knee joints were also taken. The presence of degenerative arthritis was defined as having stage 2 or more of the Kellgren-Lawrence grading system1957):13. Radiographic appearance of knee joints, the patients was categorized into four stages according to KL staging system (Kellgren and Lawrence.

Kellgren and Lawrence grading system for OA:

1-stage 0- Normal

2-Stage 1-Doubtful narrowing of the joint space and possible

Osteophytic lipping

3-Stage 2-absolute osteophytes and possible decreasing in joint space.

4-Stage 3-Moderate multiple osteophytes, definite narrowing of the joint space, some sclerosis and possible deformity of the bone contour

5-Stage 4- Large osteophytes with marked narrowing of the joint space, severe sclerosis and definite deformity of the bone contour.

Generalized degenerative arthritis was outlined as the coincidental presence of radiographic changes of knee and hand degenerative arthritis.

Laboratory investigations

Blood tests were permitted to cluster and after that centrifuged at3000 rpm for 30 minutes to get serum uric acid levels were evaluated by spectrophotometer strategy

The serum uric acid qualities were arranged into tertiles (low, normal. Abnormal states), (2, 5-6) mg\dl. Low less than 2.5 mg\dl while abnormal state more than 6 mg\dl.

Also, balanced for age, sex, knee OA and generalized OA, utilizing the Q square investigation strategy.

Findings

A number of 288 patients involved in this cross sectional study their ages ranged from (45-90) mean age 67.5 years; female patients were 216 (75%). Mean age of females 62.5. Male patients 72 (25%), mean age of males 67.5. the study shows highest rate of knee OA patients and generalized OA (76.21%,63.16 respectively) have normal level of uric acid ,while only low rate of knee OA patients and generalized OA(21.93%,31.58 respectively)have high level of uric acid which was NS (P=0.547), Table:2.

In this study we found that 6 female patients (2.78%) has low level of uric acid and 180 female patients (83.33%) have normal level .and 30 female patients (51.39) have high uric acid level. while 35 patients (48.61%) of male have an high level of uric acid and 35 male patients (48.61) have high level of uric and no

one of the have decreased level. The result was highly significant (P: 0.0001)(Table-3)

The study shows that the highest mean level of uric acid was observed in male patients comparing with females (6.087±0.190 V.S 4.577±0.81 mg/dl) with highly significant relation(Table-4).

In Table 5, there was no significant relation between knee O A and generalized O A regarding the sex and 94.44% of males have knee O A and 93.05% of female have knee O A. BMI ≤30was observed in 101 Knee OA (91.1%) and 9generalized OA (8.9%). BMI>30was in 178 knee OA (95.2%) and 9generalized OA (4.8%).P value=0.17 NS .table 6.

Table 1: Classification of body mass index (BMI) by WHO.

BMI	**Classification**
<18.5	underweight
18.5-24.9	normal
25.0-29.9	overweight
30.0-34.5	Obesity(class I)
35.0-39.9	Obesity(class II)
>40	Extreme obesity (class III)

Table 2. Relation of knee O A and generalized O A with uric acid level.

Sex	**No.**	**Uric acid level (mg /dl)**						***P.* value**
		Low		**Normal**		**High**		
		No.	**%**	**No.**	**%**	**No.**	**%**	
Knee O A	269	5	1.86	205	76.21	59	21.93	0.547 (non significant)
Generalized O A	19	1	5.26	12	63.16	6	31.58	

Table 3: Distribution of uric acid level in O A patients according to their sex.

Sex	**No.**	**Uric acid level (mg /dl)**						***P.* value**
		low		Normal		High		
		No.	%	No.	%	No.	%	
Male	72	0	0	37	51.39	35	48.61	0.0001 Highly significant (HS)
Female	216	6	2.78	180	83.33	30	13.89	

Table 4: Relation of uric acid mean level with sex

	Sex				*P.* value
	Male		Female		
	No.	Mean ± S.D	No.	Mean ± S.D	
Uric acid level (mg/dl)	72	6.087±0.190	216	4.577±0.81	0.001 (HS)

Table 5: Relation of O A status with sex

Sex	No.	Knee O A		Generalized O A		*P.* value
		No.	%	No.	%	
Male	72	68	94.44	4	5.56	0.918 Non significant
Female	216	201	93.05	15	6.95	

Table 6: shows the relation between BMI with knee OA and generalized OA.

BMI (kg/m^2)	No.	Knee O A		Generalized O A		*P.* value
		No.	%	No.	%	
≤ 30	101	92	91.1	9	8.9	0.17 (NS)
>30	187	178	95.2	9	4.8	

Discussion

In this cross sectional study we found no relationship among hyperurecemia and knee O A or generalized O A as reported before hand [3,5]. Additionally Palazzo al [6] discovered that the elements, including: diabetes mellitus, ischemic coronary illness, hypertension, physical action, conjugal status, serum uric acid, triglycerides, cholesterol and blood glucose levels) did not correspond to radiographic O A , Howard [10] inferred that (serum uric acid dimension not related with OA of knee in men). Iqbal et al [11] found in study (In all classes of knee OA serum uric acid was no significantly increased). We believed this no relationship between uric acid dimension and knee OA could be because of high serum uric corrosive in patient will prompt arrangement of uric acid crystals in the joint along these lines quicken the advancement of gouty joint inflammation which is an inflammatory arthritis, while knee OA is the most well-known type of non inflammatory joint diseases.

Different studies found a conceivable relationship among hyperurecemia and knee OA. Al-Arfaj *et al* [13], Anderson *et al* [14], Ding *et al* [15], in their studies presumed that serum uric focus and predominance of hyperurecemia are emphatically connected with OA of the knee in the female population. We additionally found a relationship between knee OA and obesity as announced already by Srikanth *et al* [16] noted positive associations of uric acid concentration with OA, but these associations became insignificant when body mass index (BMI) was controlled as confounder. On the other hand, uric acid levels at the highest tertile may be associated with OA. In a cross-sectional study, the highest serum uric acid tertile were associated with generalized OA in subjects who had undergone arthroplasty for hip OA, but not with those who had undergone arthroplasty for knee OA [1-2]. We found higher rate of knee OA among female patients as revealed beforehand by Srikanth *et al* [16] indicated that there is more serious hazard in females for common and episode knee and hand. There

was no connection between radiological evaluations of knee OA and serum uric acid dimension. Another cross-sectional study involving over 4,000 participants in China has shown that women with the highest tertile of uric acid showed the highest degree of radiographic features suggestive of knee OA after adjusted for various confounding risk factors including BMI [17]. Bevis *et al* [17] sowed in a multivariable logistic analysis model was applied to test the target associations after adjusting a number of potential confounding factors. Ma *et al* [18] he prevalence of OST was increased in the highest tertile of uric acid compared to the lowest in female subjects

Conclusion

This cross sectional study conclude that there was no association between hyperurecemia and knee degenerative arthritis nor generalized degenerative arthritis ,our suggestion for more studies to found a correlation of presence of uric acid crystals in synovial fluid of knee joints with degenerative arthritis .

Conflict of Interest: Non

Source of Findings: Self findings.

Ethical Clearance: This research was carried out with the help of my colleagues mentioned with me. The oral and paper approval of the patients in this study, which included the withdrawal of blood samples from them and laboratory tests.

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Relationship Serum Thyroid Stimulating Hormone with Body Mass Index in Patients with Thyroid Disorder

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Abstract

Hypothyroidism and hyperthyroidism have clear effects on body weight. In this study, we aimed to find the possible association between the serum thyroid stimulating hormone (TSH) and the body mass index (BMI). The weight, height and (TSH) were estimated for a total of (150) subjects whose ages ranged between (30-60) years during the period from March 2018 to July 2018. Participants were divided into three groups according to the BMI values: Normal weight (18.5-24.9Km/m^2), overweight (25-29.9Km/m^2) and obese (BMI $\geq$ 30Km/m^2). The normal value for TSH was (0.25-5µIU/ml). Levels of serum thyroid hormones were assayed by Chemiluminescence immunoassay at the laboratory of Baghdad teaching hospital/Baghdad. A significant difference (P<0.001) was found in our study between TSH value and increased BMI. Subjects with higher (BMI) had also higher TSH, and this trend continued from normal weight to obese individuals. The mean (TSH) for normal subjects was (0.7093±0.384 mIU\L), for overweight was (0.7316±0.7309 mIU\L) and for obese was (1.2752±0.8258 mIU\L). The mean serum (TSH) concentrations demonstrated a significant increasing trend with the increasing (BMI) (p<0.01). It can be concluded from these findings that mean serum TSH levels increased with increased BMI values. To confirm the relationship between thyroid hormone levels and BMI, further large scale studies are required.

***Key words**: Body mass index (BMI),Overweight; Obesity, Thyroid stimulating hormone (TSH).*

Introduction

Obesity has been increasingly identified as a main global raising health problem. Several previous studies were performed regarding the association between body mass measures including (BMI) and thyroid profile, but they were not fully understood. There was a continuous conflict between the majorities of studies on the results of this association. The BMI profile can be influenced by even a small alteration in the TSH values [1]. In euthyroid individuals, significant positive correlations are found between BMI and TSH values [2,3]. However, no findings were revealed by other studies to confirm the relationship between BMI and thyroid status [4]. The question that jumps into our minds is how to demonstrate the TSH level of people in regard to their BMI , and how to support and prove this association on scientific basis among our studied population. The major aim of our study is to show the relationship between BMI and TSH levels among healthy adult people whose TSH range between 0.4 mlIU and 10 mIU.

Thyroid gland is stimulated by a pituitary hormone called thyroid stimulating hormone, which is also known as (thyrotropin or thyrotropic hormone or TSH) to form thyrotoxin or (T4) and then triiodothyronine or (T3) that, in turn, stimulates most body tissue metabolisms [5]. TSH is a glycoprotein hormone, which is made and released by thyrotrope cells of the anterior pituitary gland to regulate thyroid's functions.

Thyroxin or (T4) is converted into triiodothyronine or (T3), the active hormone that plays a key role in stimulating metabolism. Nearly (80%) of T4 is converted into T3 in the liver and other organs, while (20%) is converted in thyroid gland itself [5]. There is a continuous secretion of TSH throughout the life, but it reaches its highest levels during the times of rapid development and growth. The thyrotropin releasing hormone or (THR) is

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produced by the hypothalamus, which is located in the brain base, and the pituitary gland, in turn, is stimulated by THR to produce TSH.

The hypothalamus also produces somatostatin hormone, which has an inverse effect on the production of TSH by pituitary gland, leading to a decrease or a reduction of its release.

The levels of serum T4 and T3 control and adjust the TSH production and release by the pituitary gland. When the levels of T4 and T3 are low, there is an increase in TSH production, whereas when the levels of T4 and T3 are high, there is a decrease in TSH production, which represents a negative feedback loop [6].

Tertiary or central disease or TSH to THR pathology may be indicated by any improper measuring of thyroid values e.g (low to normal TSH together with low to normal T4). Euthyroid sick syndrome is indicated by high reverse values of T3 (RT3) along with normal TSH and low and normal T4 and T3 levels, which may also be investigated for chronic sub-acute thyroiditis.

With a previous diagnosis of an auto immune thyroid disease, this may always be suspected to develop into SAT even when normal TSH exists, as no recovery has been yet found from autoimmune diseases.

It is necessary to state that the secretion of TSH is based on a pulsatile manner, in order to interpret the clinical laboratory results [6][7][8], leading to both ultradian and circadian rhythm of their serum levels [9].

[10] Showed that fine tuning between energy intake and consumption regulates the body weight. The factors which determine energy consumption involve: resting energy expenditure, no activity of exercise as well as the voluntary physical activity. T3 is responsible for about 30% of resting energy expenditure, and plays a key role in the homeostasis of temperature of human beings. The resting energy expenditure may also be affected by T3 via spontaneous motor activity regulation.

Subjects and methods

Our study was conducted on 150 adult subjects (59 males and 91 females). The study was performed in Baghdad teaching hospital / Baghdad city, during the period from March 2018 to July 2018. Serum thyroid stimulating hormone (TSH) was used to assess their thyroid disorders, and BMI was used to measure their body weights. Being an Iraqi adult was the only inclusion criteria for an individual to be enrolled, while exclusion criteria included any present or previous thyroid disorder, cigarette smoking, and chronic renal or hepatic diseases, taking drugs that change serum TSH levels such as metformin in addition to pregnancy. A data collection form was prepared including age, gender, height, weight as well as the laboratory results of TSH estimation. Before enrollment in the study, all participants were informed about its methods and objective. Written informed consents were taken from all persons who were told that their participation in the study is entirely voluntary. The weights in Kilograms) and the heights in meters were measured, while the participants were barefooted and putting on light clothing. The body mass index (BMI) was calculated by dividing the body weights in Kilograms by the heights in meters, and the grouping of the participants was done according to their BMI in the following form: Normal weight (18.5-24.9Km/m^2),overweight (25-29.9Km/m^2) and obese (BMI $\geq$ 30Km/m^2) [11].

Serum samples were analyzed immediately by Chemiluminescence immunoassay at the laboratory of Baghdad teaching hospital. Results of serum thyroid hormones of the participants were compared with their BMI grades. For data entry and statistical analysis of results, the statistical package of social science (SPSS version-22) program was applied, and the appropriateness of significance (ANOVA or chi square) were used for the descriptive statistics.

Findings

The current studied showed the correlation between the mean body mass index (BMI) and the mean serum (TSH) among the studied group. Table (1) indicated the distribution of gender percentage according to (BMI) among participants as follows: In the normal weight whose (BMI) ranged between (18.5-24.9Kg\$m^2$), the result of gender distribution was 11 males (18.64%) and 18 females (19.78%) , while in the overweight whose (BMI) ranged between (25-29.9 Kg\$m^2$), the result of gender distribution was 20 males (33.89%) and 23 females (25.27%), whereas in the obese group whose (BMI $\geq\geq$ 30 kg/m^2), the result of gender distribution was 28 males (47.45%) and 50 females (54.94%). It was shown that the obesity was significantly higher among females than male participants.

Table (2) and figure (1) showed the mean (TSH) values and the standard deviation (±±SD) for every (BMI) group. The mean serum (TSH) for the normal weight group was(0.7093 ±(0.7093 ± 0.384mIU\L)0.384mIU\L) , while for overweight group was (0.7316 ± 0.3709mIU\L), (0.7316 ± 0.3709mIU\L), and for obese group was(1.2752 ± 0.8258mIU\L)(1.2752 ± 0.8258mIU\L). The mean serum (TSH) concentrations revealed a significant increasing trend with the increasing (BMI) values (< 0.01).

Table (1): Distribution of body mass index (BMI) according to gender

BMI	Male (59)		Female (91)		Total	%
	N	%	N	%		
Normal weight (BMI: 18.5-24.9 kg/m²)	11	18.64	18	19.78	29	19.33
Over weight (BMI: 25-29.9 kg/m²)	20	33.89	23	25.27	43	28.66
obese (BMI ≥≥ 30 kg/m²).	28	47.45	50	54.94	78	52

Table (2): Distribution of (TSH) according to (BMI)

BMI	N	TSH mean	Standard Deviation ±SD	P-value	
Normal weight	29	0.7093	± 0.3842	0.15	NS
Over weight	43	0.8612	± 0.3709	0.005	*
obese	78	1.2752	± 0.8258	0.001	**
Total	150				

NS:- non significant, *:-significant ≤ 0.05 , **:- highly significant ≤ 0.01.

A great deal of attention was given by medical researchers to the relationship between BMI and thyroid hormones in euthyroid adult people [12]. Thus, the aim of the current study was to measure serum thyroid hormone levels in Iraqi adults and correlates them with their (BMI). In this study, the results showed that the majority of participants fall within overweight (28.66%) and obese (52%) categories, and there was a highly significant obesity conditions in females than males.

These results were in agreement with the study of [13] who found a significant correlation between mean TSH levels and BMI. Our results demonstrated that serum TSH levels of the studied individuals recorded a highly significant trend with the increasing (BMI).

Other researchers such as [14] found no relationship between serum TSH levels and free thyroxin levels within the normal BMI range when they studied the effect of thyroid hormones on BMI. In contrast, other researchers such as [1] reported that thyroid function may be among those factors which function in conformity for body weight determination.

Chan *et al* [15] clarified the TSH-BMI relationship to be under the adipose tissue signal impacts, and they also indicated the possible significant effect of leptin on the central regulation of thyroid function via TRH. [16] Hypothesized that the positive association between serum TSH and leptin levels is also positively associated with the TSH-BMI relationship.

High triglyceride (TG) and low high density lipoprotein (HDL) levels were detected in subclinical hypothyroidism patients by [17].

Furthermore, [18] showed the presence of a positive relationship between (TSH) and (BMI), assuming the possibility of presence of other subclinical hypothyroidism condition, i.e elevated (TSH) concentrations, although the serum concentrations of the hormone are normal.

However, whether the verification of elevated serum TSH to be the cause of the influence is certainly confirmed or not, A. The [19, 20] observed that thyroid function disorders could be the primary and BMI alterations could be the secondary cause and vice versa.

The well-established receptor expression and the message transfer inside the adipocytes affirm the biological significance of the positive association between obesity and serum TSH levels. The possible existence of ''Hypothalamus-Pituitary-Adipocyte axis'' is proven when the expression of multiple pituitary hormone receptors is considered. In regard to downward regulation, the association between obesity and TSH levels may be stable. In addition, a system of feedback regulations may be necessary for this axis, therefore, the positive relationship between obesity and TSH may be reversely interpreted [21]. There is an association between weight gain and thyroid gland as thyroid gland releases hormones which help in metabolism regulation. This process controls the body utilizing of energy, when an individual may feel cold, tired or stagnant. Human's body is clogged by salt and water due to hypothyroidism, which in turn leads to body swelling [22, 23].

Conclusion

It can be concluded from this study that there was an elevation in the levels of mean serum TSH when the BMI increased. Additional large scale studies are recommended to confirm the correlation between thyroid hormone concentrations and BMI values in euthyroid adults.

Source of Funding: - Self

Ethical Clearance: Formal administrative approval was obtained from laboratory of Baghdad teaching hospital.

Conflict of Interest:-Non

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The Long Term Outcome of Graft Urethroplasty by Buccal Mucosa in Penile and Bulbar Stricture

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Abstract

Background Urethral stricture is widely prevalent disease and its management is a challenging surgery. Nosoleideal surgical procedure appropriate inevery situation. The Grafting by Buccal Mucosa (BMGurethroplasty) represents the most appropriate and widely used procedure torepairstricture of urethra. We disclosedtheextended followup thecasesof stricture of urethrathat managed by (BMG urethroplasty) aiming to determine the success rate, recurrence and complications rate. **Patients and Method** A study was orchestrated to prospectively followup a 39 patients with bulbar and proximal penile urethral stricture disease repaired by BMG urethroplasty. Retrograde urethrography done to confirm the diagnosis , measure the length and locate urethral strictures. Patient demographics, stricture aetiology and anatomy all were recorded. Follow up data in term of success and failure rate, and complication. **Results** thirty-nine patients (the mean age was 35.4 years) with urethral strictures disease diagnosed by urethrography of more than 3 cm in length(the mean ofstricture length was 5.34cm). 23, 7, and 9 patients had bulbar, penile and long combined (bulbar and penile) strictures respectively. Early peri- and post-operative complications occurred in 35% it was almost minor complications included scrotal swelling, minor hematoma, urinary infections, and urgency. 4 patients only developed re-stricture 2 of them had a failure. The most frequent major complications were a urethrocutaneousfistula. **Conclusion** Buccal mucosal graft urethroplasty appear to be a rightful and durable treatmentchoice for cases with urethral strictures, moreover it has a fewer major complications.

Keywords: *Buccal mucosal graft (BMGurethroplasty) Urethra, spongiofibrosis, stricture.*

Introduction

Urethral stricture is a most prevailing cause of snag of lower urinary tract in the males between 3rd and 5thdecade, carrying an evaluated overall prevalence of 0.5%. Stricture of urethra is an aberrantdiminished diameter of the urethra, that due to a varying degrees of spongiofibrosis, with 50% confined to the bulbar urethra, and 20% penile part[(1)]. We can broadly categorize the causes of strictures in urethra into trauma, infection and inflammation[(1)]. Sexually-transmitted diseases (mostly gonorrhea), result in chronic bacterial urethritis and responsible for up to 20% of cases, while 30% of urethral strictures are considered idiopathic [(2)]

The presenting features tend to be related to obstructive voiding symptoms, with a poor urinary stream, post void dripling and a perceivean incomplete evacuation of bladder, or symptoms of recurrent urinary tract infections [(3)]. Non-invasive investigative methods such as uroflowmetry, the results of which produces a pathognomonic curve with a prolonged voiding time and low-level flow, and ultrasound post-void residual measurements will demonstrate reduced bladder emptying.

Endoscopic visualisation via cystourethroscopy can identify the location of the stricture but provides minimal information on stricture length. Ultrasonography is useful in demonstrating depth of spongiofibrosis, whilst contrast studies such as retrograde urethrography and voiding cystourethrography accurately demonstrate the anatomical location and length of the stricture, which guides further treatment[(4)]

The Traditional management of urethral stricture was by direct visual urethrotomy, however the reported recurrence rates are as high as 60%.[(5)] Poor long-term

outcomes from these endoscopic methods have presented an opportunity for open reconstruction (urethroplasty) to develop[6]

Urethroplasty is a surgical operation that aimed to reconstruct or repair the urethra. Many surgical techniques used nowadays, but the most commonly practiced are anastomotic and augmentation urethroplasty, the latter using either buccal or lingual mucosal grafts [7]Differing types of autograft tissues have been described, with buccal or sublingual mucosal grafts (OMG),penile and scrotal skin [8], bladder mucosa, colonic mucosa[9] and tissue engineering of grafts [10].

First described by Humby in 1941[11], BMG urethroplasty is now widely used in contemporary practice. The donator area left for healing by the mean of secondary healing, whichmends successfully [12], but we don't achieve that an improvement in postoperative pain and oral intake that can be seen with primary closure [13].

Postoperative complications of urethroplasty include swelling and bruising around the wound, numbness or discomfort around the buccal mucosal graft donor site or spraying of urine (all up to 50% of patients). Less common complications include wound infection, erectile dysfunction (up to 10%), anastomotic leak and urinary fistula (around 2%).Stricture recurrence is a major complication, with reported rates of up to 14% for anastomotic and 42% for augmentation / substitution urethroplasty, after long-term (more than 10 years) follow-up [14]. However, through comparison with stricture recurrence rates in other treatments (up to 60% in urethrotomy[5]), it is apparent that urethroplasty offers superior results in terms of restricture.

Patients and Method

With this prospective study we aimed to follow 39 casesofproximal and bulbar penile stricture disease of urethra and to determine the long term result of their repair by using BMG urethroplasty which was conducted at Tikrit city from April 2007 to December 2018 with a followup duration mean of 28 months.

Pre-operative evaluation includes a detailed medical and surgical history as well asclinical examination aimed at detecting the etiological factors such as instrumentation, prostatectomy, urethral catheterization, TURP, trauma, and urethritis. Pre-operative evalution also include culture of urine, postvoiding residual volume of urine measured by ultrasonography, as well uroflowmeter to evaluate urine flow rate. Patients evaluated with a combined retrograde urethrography and micturatingcystourethrography. This helps the determine of the site, length, and multiplicity of the stricture (although thechoice of the surgical technique do not influenced by stricture causenor length).

The procedure done under general anesthesiathrough a midline perineal incision. Division of bulbocavernosus muscle through the midline exposes theanterior urethral corpus spongiosum, which is the part affected by spongiofibrosisand lead to narrowing of the urethra. The identification of the stricturedpartbegins at the tip of the catheterincerted within the urethra,whichincised dorsally. The incision then extended proximally distal to the end of the stricture until healthy spongiosal tissue is experienced. An adequate lengthgraft of buccal mucosa obtained from the interiorcheek just below the duct of Stensen's. Up to 10 cm in length and 3.5 width can be obtained, the extra length gained by extending the dissection into the tonsillar fossa posteriorly and to the lower lips anteriorly. Infusion of the buccal area with (1% lidocaine with 1:100,000 epinephrine to a volume of 5 to 10 cc) may help to reduce the hemorrhage as well as the graft will be elevated and adissection plane will be provided.

Tailoring of the graft to a proper size is done after defatting. The lumen of distal andproximal urethraare assessed. The graft fixed to the dorsum using few 4-0 vicryl sutures forsupport and reinforcement as well toocclude the dead space. Insertion of 18F silicone, or silicone coated Foley catheter through the urethra into the urinary bladder as a cast of healing and for drainage. Urethra is then rotated back to its position and the mucosal margin of the urethra sutured to the free margin of the graft. Drainage by suprapubic catheter is obsolete. Cover with antibioticsare continued until the catheter is removed usually after3-4 weeks. Three months later, after removal of catheter, flow rate and retrograde urethrography is done. Uroflowmetery with normal flow rate is regarded as a successful resultswhilecases with lowflow rate are referred to (Direct Visual Internal Urethrotomy(DVIU)), accepted voiding rate after onetrial of DVIU is regarded as a succeeded procedure . A recurrent weak stream after even this single trial of DVIU will be considered as failed treatment.

Results

A thirty ninecases of different age groups(the mean 35.4 years) with urethral strictures disease diagnosed by urethrography. Single session dorsal reconstruction of urethra usingBMG was performed toevery one of these cases with indwelling silicone Foley's catheter removed after 3-4 weeks with a mean followup time for themwas 28months(Fig. 1).

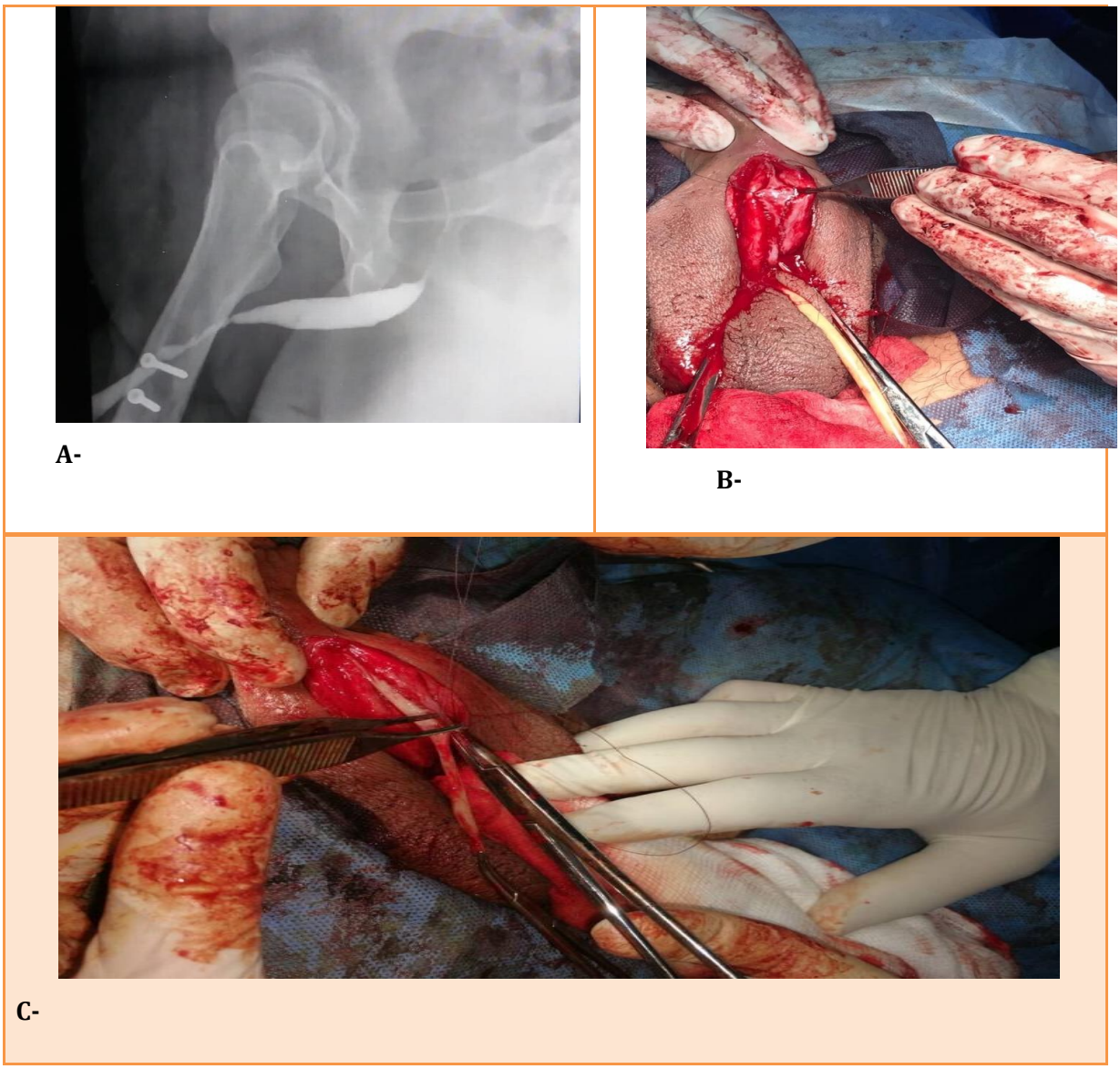

Figure (1): Single stage dorsal BMG reconstruction of urethral stricture. A) urethrography shows narrowing of the anterior part of urethra. B) Penoscrotal incision. C) Dissection of corpus spongiosum and fixation of graft.

Complication at the donator site occurred only in one of the cases with infection and ulcer that took up to one month to heal completely. Although swelling of the face, numbness and limitationofthe opening movement of the mouth are uncommon, though they are fade uneventfully. Early peri- and post-operative complications occurred in 35% it was almost minor complications included scrotal swelling, minor haematoma, urinary infections and urgency. 4 patients only developed stricture again at the site of operation 2 of them got good stream after a trial of DVIU, the other 2 patients did not responded and required another urethroplasty and regarded as a failure of the technique (failure rate 5.1%). The most frequent major complications were urethrocutaneous fistula that occur in 6 patients(15%) mainly those with involvement of penile urethra. Urinary infection

happened in 10 patients and responded well with good antibiotics directed by the results of urine culture. Erectile dysfunction and mild chordee noted in few patients. Improvement in the Peak urinary flow rates was dramatically noted postoperatively (range 18.2 to 24 ml/sec) as compared with preoperative data base of a range 0 to 9.3 ml/sec(see table 1 and 2) .

Table(1): complication of BMG

Complications	No.
Long term Recurrence 2	
Urethrocutaneous fistula	6
Wound of infection	4
Chordee	2
Erectile dysfunction	2
Facial Swelling	9
Numbness	7
Limitation in mouth opening	7

Table 2: Etiology behind strictured urethra.

Etiology	**No.**
Urethritis (infection)	9
Iatrogenic	8
Idiopathic	15
Injury (blunt)	5
Shell injury	2

Mean of the extent of the stricture, as spanned by preoperatively done RGU was 5.34 cm (all of it was more than 3 cm). Twenty three of cases sustained bulbar stricture, while only seven had proximal part of the penile urethra affected,meantime theinvolvement both areas synonymously was faced in 9 patients(table 3).

Table 3: the location of the strictured part of urethra.

Location	Number
bulbar	23
Bulbar-penile	9
penile	7

Discussion

Humby in 1941 was the first described graft urethroplasty using buccal mucosa.[15] Due to itsglowingfeatures that made it typical urethral deputy, as iteasilygleaned, easily handledsurgically , glabrous, moist environmentaccordance, and paramountearly ingrowth and uptake[16] . However, adisputation raised whether the graftpositionmust be ventrally or to the dorsum. The dorsal place is preferred by most of experts, however many trials shown that the dorsal place of grafthas a generous blood supply and back brace as theventralonlayplaced one. The rateof succeeds was of no significant difference according to Barbagli et al, who involved a fifty cases in their study and the graft was placed ventrally, dorsally or laterally on the urethral surface. The dorsal BMG on-layannounced a 85 and 100 %prosper rate[17].

In our present series, Dorsal BMG urethroplasty has a flourished succeed rate of 94.9 % with followupmeantime of 28months. Elliott SP et alseries published in the 2003 [18] who escortedsixtycases of buccal graft reconstruction with mean followup of forty seven months, announced a 97% prosper rate after single attempt of internal visual optical urethrotomy. These results underpinned by Kane CJ et al. [19] who reported disclose a 94.3 % successful results in their series with a mean followup of twenty five months postoperatively. Iselin & Webster published a study at1999 in which they accomplished29 dorsal on-lay graft urethroplasty, and describe a soaring early successfulresults reaches up to 97%, at a median followup of 19months, but they emphasize that a long time of followup is required as the succeeded urethroplasty operation is spaned in decades[20].

In general, complications of BMG urethroplasty are uncommon . Pitfalls occurred as a postoperative complications can happened in 2 areas, the donor area and the repair site in the strictured urethra.[21] Potentially buccal mucosal pick up site complications encompassed facial swelling, bleeding, discomfort and pain, numbness and paresthesia, limitationof movement in mouth opening and damage to the duct ofStensen. Facial edema and limitation in mouth opening are frequent, though they are selflimiting and usually resolved within a couple of months after operation[22]. Wood et al, reported that closure of the donator site was followed by awful complains and nasty pain, this

make him to suggest that this may be ameliorated by left open.[22]In contrast Dublin et al. announced that a preponderance of casesshowed a good responce with the primary suturing of the pickup site, and they disclose that restriction in mouth openingwas the most frequent long-term complaint, and occurred in 32% of cases, and half of this number hadnumbness. In general, the interior cheek pickup site for BMG appears to heal without long-term pitfallsregardless of management[21].

Complications of implantation site at perinium are asuncommon as donor site complications. Urethro-cutaneous fistula, re-stricture, local infections of perineal wounds or urethral anastomosis site , hematomas, and paresthesia of the skin, do sometimes occur. Fichtner*etal.*,[23] found in their study that an all-inclusiverate of complications was 25%. In general the complication rate in other study[24]was found to be 5.4%, of them 5.7% had a re-stricture rate while in our case series we faced ageneral complication rate of about 18.6 % which include urethrocutaneous fistulae, re-stricture, urinary tract and local wound infection.

Conclusion

Regardless of the etiology, length and site of urethral stricture disease, BMG urethroplasty seems to be the prime surgical procedure referred to its high rate succeed, low recurrence rate, as well as infrequent and short standing complications if compared with other techniques. It is picked up easily and unchallenging to be handled. It also resist infections and withstand the moist environment. We used graft urethroplasty with buccal mucosa victoriously for managinglong strictures in urethra, with fewer complications and few donator placerate of morbidity.

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Hardness and Roughness of Flexible Denture Base at Different Injection Time and Different Temperature

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Abstract

Most problems with heat cured acrylic are difficult to address, in recent time flexible dentures have become more popular, although the search for the ideal option is still on, so this study was conducted to study the effect of different temperature and different injection time to study the effect on the hardness and roughness of flexible denture. 60 specimens from metal pattern were prepared with dimension (60mm, 10mm, 3mm) length, width and thickness respectively. Specimens were allocated into two group;30 specimens for surface roughness measure(groupI)and 30 specimens for hardness measure(group II),these two group subdivided into three subgroups according to time of injection and temperature (10 specimens for each subgroup), group A: injection at (270◦C) in (14 min.) (control). group B: injection at(300◦C) in (10 min.) and group C: injection at (250◦C) in (24 min.). For hardness measure all specimens were tested by (shore D device DINISO electrometer). All specimens were tested for roughness measure by surface roughness tester (profilometer , digital) the result expressed in micrometer. Result showed there were no significant differences in hardness and roughness measures between both group B and group C with control group (A) P0.05. The max. mean value of roughness was recorded by group(A)which was the control group and group (B) recorded the min. mean value of roughness. While for the hardness the min. mean value was recorded by group(B),while group(C) recorded the max. mean value of hardness, however there were no significant different(P0.05) between three groups A, B and C both in hardness and roughness measures.

Key Words *:flexible acrylic hardness roughness injection temperatures*

Introduction

One of the most important needs for patients attending clinics to restore esthetics and/or function is the replacement of missing teeth, for replacing missing teeth many treatment modalities are available.

The choice between many treatment options is influenced by clinical, dentist and patient immanent factors[1].Excellent alternatives to conventionally methyl-methacrylate dentures are flexible denture, which not only provides excellent aesthetics and comfort, but also adapt to the constant movement in partially edentulous patients [2]. adapt well in the undercut areas and not cause sore spots. They also have almost no porosity and lower elastic modulus [3]. Furthermore, the aesthetic of removable partial denture with heat cured acrylic, may be compromised by the visibility of metal clasps [4], however, reported cases of intolerance to monomers present in acrylic materials among patients and medical staff have been increased[5,6].

The increase in processing temperature under pressure during polymerization of acrylic materials could result in a more complete polymerization reaction and thus producing a harder polymer network[7,8and9]**.** but no study concerning the effect of increase temperature on flexible acrylic material

Since the surface roughness is an important factor because rough denture surfaces can cause biofilm formation, fungus and bacteria have more propensity to adhere to rough surface so accumulation of microorganisms affect oral health [10] and the hardness test has been used for characteristic of the mechanical quality of polymer [11], so this study was performed to

evaluate the hardness and surface roughness of flexible denture material at different injection time and different temperature.

Material and Method

Flexible denture base material

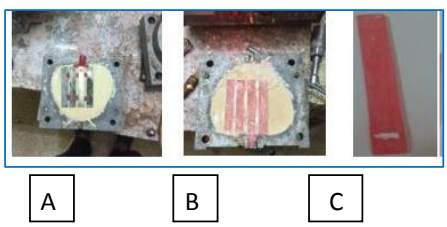
The material was available in the form of granules in cartridges (Lingchen dental /china)as seen in(Fig.1) the cartridges contain thermoplastic grains are heated to plasticize the resin. All material were suited for the thermoplastic process (injection thermo press machine / china) by injection molding technique the resin material were injected under very high pressure.

Fig. (1) sample of cartridges

Preparation of mould:

Total sample were 60 specimens all sample were prepared in the form rectangular metal shaped pattern according to ADA Specification NO.12(1999) [12] the dimension (65mm*10mm*2.5±0.1mm) length, width, thickness as seen in (Fig.2) were constructed to be used (30 samples) for indentation hardness and (30 samples) for surface roughness.

The preparation of mould was done by conventional procedures and the wax elimination was done by using boiling water, , after that flask was opened for cooling (at room temperature),the flexible denture base material was injected in the electrical furnace (injection thermo press machine Lingchen dental /china)according to manufacture instruction and study design ,then left to cool at room temperature. The conventional flasking, packing procedures were followed in the preparation of the specimens [13].



Fig(2) Flexible acrylic resin samples: A-Metal sample in the flask B-Wax sample in the flask C-Flexible acrylic sample

Distribution of the sample

60 specimens divided into two groups according to measuring test (30 specimens for each group), group I for surface roughness and group II for indentation hardness, both groups were subdivided into 3subgroups A, B and C(10 samples for each subgroup)according to injection time and temperature.

Group A: injection 10 samples at (270◦c) in (14min) (control that inject according to manifuctural instructions).

Group B: injection 10 samples at (300◦c) in (9 min).

Group C: injection 10 samples at (250◦c) in (24 min)

Finishing and Polishing: Take the specimen of flexible and remove grass of excess resin from the border by large acrylic burs until make specimen smoothing is. The polishing done by pumice with water and store it in the plastic container of water until needed.

Measurement of surface roughness:

The surface of the test specimen was analyzed with surface roughness tester (Profilometer-Digital, China, TR200)(Fig3) to study the micro geometry of the test surface. The diamond stylus of the profilometer was moved about 4mm across the surface of the acrylic specimen. According to the manufacture instructions of the device, the vertical displacement of the stylus is measured as the surface variations, usually measuring from 10 nm to 1mm ,the height position of diamond stylus is converted to a digital signal ,which is stored and displayed a(2mm) distance separated each reading and all measures were carried out by same researcher. three readings were recorded for each specimen and the mean value for each specimen was the average of three readings, all the specimens were examined after finishing & polishing. The results were expressed in micrometer.

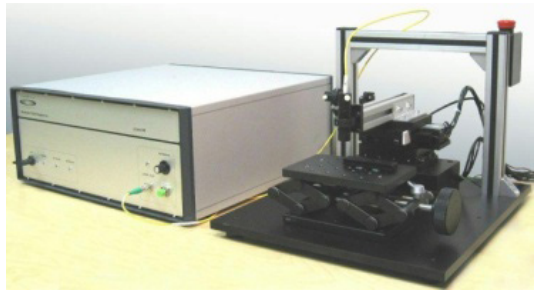


Figure (3) Profilometer device for measuring surface roughness Measurement of indentation hardness:

Shore hardness tester was use in this study for measuring the indentation or measuring the hardness of the specimens (DINISO 7619electrometer/Germany) (Fig4){according to ASTM D2240-03 standard}[14]. The Shore D hardness device was vertically placed over flat sample supported by flat, rigid base. The readings were taken after three second of stable contact over specimen, when indenter was pressed down quickly and firmly on, to record the maximum reading. The reading was taken directly from the reading scale The contact surface of the shore hardness tester must be parallel to the specimen support of the test stand to prevent error in measurements. The distance between the specimen surface and the indentor of the hardness tester to be 5-12 mm. During carrying out the test the contact was set period between the specimen and the indentor was 6 seconds. For each specimen, 5 points were marked with 6 mm distance between each other, the hardness value was calculated and the average of these five reading After the measurements were taken directly from the scale reading was calculated.

(A) (B)

Figure(4): (A)Shore D hardness test device,(B) specimen placed under the device

Findings

Surface Roughness Test (groupI)

Table (1): Descriptive data of groups of the surface roughness test

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group (A) (control)	10	1.4582	.24401	.08627	1.2542	1.6622	1.08	1.65
Group (B)	10	1.3589	.67422	.23837	.7952	1.9225	.74	2.31
Group (C)	10	1.3686	.26251	.09281	1.1491	1.5881	1.13	1.74
Total	30	1.3952	.42371	.08649	1.2163	1.5741	.74	2.31

Table (1) show the descriptive of groups: mean, S.D,S.E, min., and max., values of the surface roughness of all groups. The max. mean value was (1.4582) recorded by group(A)which was the control group and group (B) recorded the min. mean value of surface roughness which was (1.3589)

Table (2) ; ANOVA test between groups of the surface roughness test

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.048	2	.024	.123	.885
Within	4.081	21	.194		
Groups Total	4.129	23			

In Table (2) shows the ANOVA test between groups, there were no significant differences between groups (n-sig) $p>0.05$.

Hardness Test (groupII)

Table(3) ; Descriptive data of groups of the hardness test

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group (A) (control)	10	66.5000	.97395	.34434	65.6858	67.3142	65.20	68.20
Group (B)	10	66.4375	3.48299	1.23142	63.5256	69.3494	58.00	69.00
Group (C)	10	67.8000	.71714	.25355	67.2005	68.3995	66.60	68.60
Total	30	66.9125	2.13283	.43536	66.0119	67.8131	58.00	69.00

Table (3) show the descriptive of groups mean, S.D, S.E, max., and min. values of the hardness groups ..The min. mean value was (66.4375) recorded by group(B),while group(C) recorded the max. mean value of hardness which was (67.8000).

Table(4) : ANOVA test between groups of hardness test

	Sum of square Squares	Df	Mean Square	F	Sig.
Between groups	9.468	2	4.734	1.045	.369
Within groups	95.159	21	4.531		
Total	104.626	23			

Table(4) show the ANOVA test between groups ,there were no significant differences between groups (n-sig) p> 0.05

Discussion

Using flexible denture has increased drastically in the late decade. The excellent tissue friendly and good mechanical properties had open up a new clinical application in dentistry and since heat cured acrylic are hard material so they may be disturbing patients during use and an increasing rate of intolerance to monomers present in acrylic materials among medical staff and patients has been reported [5,6]. Furthermore, the aesthetic appearance of removable partial dentures with heat cured acrylic bases may be compromised by the visibility of metal clasps [4,15].While the flexibility of flexible allows retentive elements that match the color of the gums or teeth. Despite the sore formation in heat cured acrylic in comparison to flexible that not cause sore spot [16,17],the flexible denture appears to have a greater role to play in futures. Material (without chemical changes) is softened by heat and injected afterwards and partial dentures might be pressed in one piece including clasps, minor and major connectors, and denture bases [15]. The surface roughness is an important factor because many studies have shown there was a direct link between surface roughness, color stability and accumulation of

dental plaque as well as adherence of candida albicans [18,19]. Furthermore the irregularities of the surface act as a nucleation sites for corrosion[20]

Hardness has been widely used as a method of investigation factors that influence the degree of conversion of resin and for characterization of the mechanical quality of acrylic [21,22]. In this study both hardness and surface roughness measures of flexible denture base material were tested at different temperature and different injection time.

The result show that the all groups that injection at different temperature (300◦C), (250◦C) and different injection time (9min), (24min) respectively ,there were no significant difference between them (p>0.05) both in hardness and roughness measures when compared with control group that injection at (14min) in(270◦c) .

In this study, the injection molding system was used, its advantages that the resin is delivered in a cartridge that eliminates dosage errors, to ensure long term stability of the shape, reduced contraction, and mechanical resistance with aging. In addition, superior physical properties, more esthetic and comfortable for the patient[23] the increasing in surface roughness may be related to injection process, melted material during the injection will roll into sprue (smaller tube) until it reaches the mold cavities and during this flow movement a small nucleus will form and cause increase in the surface roughness.

For hardness the max. mean value recorded by the group(C). this might be due to higher crystallization of resin material at this time and temperature (the higher crystallinity in a flexible, the harder material will be) [24].

In acrylic material the increase in processing temperature under pressure during polymerization reaction produced a harder polymer network[9], this may related to complete polymerization reaction that occur in high temperature for optimum time which support the result in this study, in flexible material increased time with decreasing temperature lead to result that gave more hardness material as result by group C [that injected with increased time of injection and decreasing temperature (24min. in 250◦c)] and because of increase time of exposure to heat which produce greater degree of polymerization, however ,there were no significant differences between group C and group B(300◦c-9min.) this might be due to increasing the polymerization temperature lead to harder network that result in a hard material[9].

There were no previous studies found related on the properties of flexible material with changing in temperature and injection time ,in order to compare with this study. Search for ideal properties for flexible material is still on.

Conclusion

This study show hardness and surface roughness measures of flexible denture base material when tested at different temperature and different injection time(300◦c in 9 min, and 250◦c 24 min.),there were no significant differences P0.05 with control group that inject according to manifuctural instructions(270◦c in 14min).

Conflict of Interest: Non

Source of Funding: Self

Ethical Clearance: Non

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Prophylactic Antiepileptic Drugs for Intraparanchymal Hemorrhage Patients in Salah Al-Den General Hospital

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Abstract

Background: Intraparenchymal hemorrhage considers the most severe form of stroke and has a significant cause of death. There are many cases associated with seizures development. Risk factors for post-hemorrhagic stroke seizure are cortical involvement, large lesion (involving more than one lobe), lobar hemorrhage, and male sex. The post- hemorrhagic stroke seizures affect inversely on fatality rate, recovery period and neurological outcomes.the aim of the research is to assess the effect of depakin to prevent the incidence of seizure in patient with spontaneous non-traumatic, non-aneurysmal intraparenchymal hemorrhage (NTNA-IPH) and to evaluate the effect of depakine on the fatality rate. **Patients and method** the study involved 100 patients with a definite diagnosis of NTNA-IPH. Type of the study was prospective comparative, which done in Salah-Al-den general hospital. The duration period was 10 months. The cases were classified into two groups A and B, each one consist of 50 patients. Depakine in a dose of 400mg per day for 30 days was given for group A while group B didn't be given the drug, and this is a control group. The cases of each group were followed up for 6 months from the starting of attack and during follow up, patients were monitored for development of seizure. **Results** The study result represent 14.28% of patients taking depakine had been developed seizure (6 patients from total 42 patients) and about 34.10% of patients not taking depakine had been developed seizure (13 patients from total 38 patients) with a significant difference between the two groups. Partial seizures were represent about 13.75% of post-hemorrhagic stroke seizures, myoclonic seizures 5% and tonic clonic seizure 5% in total of both groups.The overall incidence of post-hemorrhagic stroke seizure in the total 80 patients was 23.75%. The study showed that fatality rate in patients receiving depakine was 12% while fatality rate in patients not receiving depakine was 24% with a significant difference between patients with depakine and patients without depakine. **Conclusion** of this study shows that NTNA-IPH founds most commonly between 45-71 years old. The incidence of seizures can be decreased by two-folds by using of depakine as a prophylactic drug in a patients with IPH and the use of depakine in the prophylaxis against post-hemorrhagic stroke seizure have a significant role in reducing the fatality rate and improving survival.

Keywords : *Prophylactic Antiepileptic Drugs ; Intraparanchymal Hemorrhage; Patients ; Salah Al-Den General Hospital.*

Introduction

Non traumatic intraparenchymal hemorrhage (IPH) is bleeding into the parenchyma of the brain that may extend into the ventricles and, in rare cases, the subarachnoid space [(1).] Each year, about 37,000 to 52,400 people in the United States have an IPH [(2).]IPH represent 10-15% of stroke and is associated with the highest fatality rate, about 38% of patients surviving the 12 months[(3).] The majority of surviving patients to hospital presentation, the prognosis remains poor, with 35% of patients dead was dying in the first seven days and 50% within thirty days.

The prevalence of seizures after ICH varies widely from less than 10% [(4,5)] to more than 20% [(6).] The prevalence of seizures after IPH depends on study design, diagnostic criteria, duration of follow-up, and the patient studied. Risk factors for post-hemorrhagic seizure were found to be IPH , SAH, cortical involvement , large

lesion (involving more than one lobe) and male sex[7]. Stroke is the commonest cause of seizures in the old patients [8].

IPH-related seizures classified as early and late-onset. Usually most of the early- seizures found in the first two weeks [9].Patients who had frontal lobe hemorrhage were high incidence to develop early as well as late onset seizures due to the presence of motor phenomena, early seizures may be result from cellular biochemical dysfunction and structural disruption, late seizures may be caused by gliosis and the development of meningocerebral cicatrices [10].

Patients and methods

Prospective study was done in salah-Alden general hospital through the period from beginning of December 2016 to end of July 2017. The patients were selected randomly from those who admitted to medical ward with IPH in all age groups and both sexes. The sample consist from100 patients with IPH. The patients involved in this study were divided into 2 part as follows:-

Group **A** consist of 50 patients with IPH who prescribed depakine in a dose of 400mg once daily for 30 days as a prophylactic drug and was follow up for 6 months to see whether seizure occurred or not during or after the attack of IPH and the follow up was by re-examination or by telephone number of the patient if he can't get coming to hospital.

Group **B** include 50 patients with IPH , who didn't be given depakine as control group, were follow up for 6 months to show whether seizure present or not. If the patient developed fit in this group, was treated by anti-epileptic drugs(AED).

(Questionnaires): Full and detailed history from the patient or witness (if consciousness is impaired) was taken regarding age, sex, address, past medical history, previous attack of IPH, drugs , previous attack of seizure or family history of seizure and whether seizure found or not before admitted to the hospital. If the patient had seizure, detailed history was taken about the seizure including time, single or recurrent, description of seizure (partial or generalized seizure) and how many times found.

Clinical examination: Patient with intraparanchymal hemorrhage through the period of study was admitted in the medical ward, neurological examination was done for every patients involved in this study. The examination included consciousness level, motor examination of upper and lower limbs, sensory examination, cranial nerve examination and examination of cerebellar signs.

Imaging study : Every patient admitted to the hospital with acute neurological features like focal signs, disturbances in the consciousness , new onset of epilepsy, etc, was sent for taking urgent native (CT) of the brain and the radiology report was made by the radiologist. The magnetic resonant angiography (MRA) was done in selected patients in whom SAH was very suggestive clinically. This was helped the study to identify and exclude aneurysmal hemorrhage or A-V malformations. According to the result of the CT study and MRA (if needed), the patient will be included in the study if the inclusion criteria met while those with exclusion criteria were excluded from the study.

Blood tests: Blood samples were taken from every case. Routine investigations were perform include complete blood count, renal and liver function tests, blood sugar and electrolytes to exclude metabolic causes of the neurological deficits in addition to bleeding time, prothrombin time and INR to exclude bleeding tendency as a cause.

Patient group & follow up: The number of patients involved in this study was 100 patients. The study started from the first of December 2016 until the end of July 2017 and every case was follow up for 6 months, so the patients selected at Decemer 2016 ,their follow up stopped at May 2017and so on. The cases were classified into two groups , group **A** and group **B**, in which group **A** patients were given depakine for 30 days and group **B** patients didn't be given the drug and each group were follow up for 6 months.

Statistical analysis and data management: The Statistical Package for Social Sciences (SPSS, version 18) was used for data entry and analysis. The associations between categorical variables and seizure occurrence during the study period were analyzed using Paired Student t test. The differences in seizure occurrence were compared between groups using Paired Student's t-test to compare means of numerical variables. P value of ≤ 0.05 was regarded as statistically significant.

Funding

Table (1) Age distribution in 100 patients with IPH.

Age/years	Number of Patients	Mean(years)	Percentage (%)
<45	20	**38.5**	20%
≥45	80	**62.4**	80%

One hundred cases were participated in the study. 56 patients were male and 44 patients were female.

Table (2) the frequency of seizures in group A and B.

Group	Number of patients	Patients develop fits	Patients not develop	Incidence of fit	P value
A	42	6	36	14.28%	
B	38	13	25	34.10%	
A+B	80	19	61	23.75%	

Eighty patients were aged over 45 years and the remainder 20 was under 45 years. Mean for aged group less than 45 was 38.5 years .The range of age was 33-45 years , while mean for aged group more than 45 was 62.4, the range is 52-78 years.

Group A : The number of patients was 42(after subtracting the dead number of patients and loss of them during follow up in this group) 6 patients (14.28%) from total 42 developed seizure in spite of taking a depakine . The patients in group **A** who didn't developed fits throughout the period were 36 cases (85.72%) and they are taking the treatment in regular period in its dose 400mg daily.

Group B: This group included 38 patients (after subtracting the dead number of patients in this group), the result show that 13 patients (34.10%)from 38 patients had developed seizures . The other remaining 25 cases (65.78%) didn't get seizures throughout the follow up. The incidence of seizures in both aged group were 23.75%, while the remainder 61 cases (76.25%) not develop seizures.

Table(3) Types of seizure seen in group A and group B

Groups	Total number of cases	Number of patients With fits	%	Partial seizure	%	Myoclonic seizure	%	Tonic clonic seizure	%
Group **A**	42	6	14.28	4	9.52	1	2.38	1	2.38
Group **B**	38	13	34.21	7	18.42	3	**7.89**	**3**	7.89
Total sample size A+B	80	19	23.75	11	13.75	4	**5**	**4**	**5**

The study result show that the most common type of fits in the acute stage of IPH was partial type, which was characterized by a focal movements in limbs or face which was described by the patients or the witness.

Group A Sixth patients (14.28%) from the 42 patients developed fits. four patient (9.52%) was partial seizure and one patient was myoclonic seizure (2.38%), and another one had tonic-clonic (2.38%).

Group B This group involved 38 patients, 13 patients (34.21%) had been developed fits. 7 patients (18.42%) developed partial fits and three patients (7.89%) developed myoclonic fits and another three patients (7.89%) were developed tonic clonic type. The incidence of partial seizures in the total number of both sample size was (13.75%), while incidence of myoclonic type was (5%) and tonic clonic type was (5%).

Table (4) Time of seizure incidence in groupA and group B.

Group	Total number	Number of fit patients	%	Number of fit cases<14 days	%	Number of fit cases>14 days	%
Group A	42	6	14.28	4	9.52	2	4.76
Group B	38	13	34.21	8	21.05	5	13.15
Total	80	19	23.75	12	15	7	8.75

Chi square =0.046; Correlation=0.049; P-value=0.08;Significant association

The result of this study show the following: the early seizure was defined as any seizure found in the first two weeks after the time of IPH, while late seizures was determined after 2 weeks, the result show as follow; the patients who take depakine developed early fits in a low incidence (9.52%) than those who didn't take the drug (21.05%). There was a significant difference in incidence of early and late seizure in group **A** and **B**.

Table (5)Distribution of death causes in group A and group B.

Causes of death	Total number cases Group A(50)	Fatality rate %	Total number cases Group B(50)	fatality rate %
Overall	6	12	12	24
Deep coma	3	6	4	8
Multiple bleeds	2	4	1	2
complications	1	2	3	6
Past attack(s)	-	-	2	4
Others eg.drugs	-	-	2	4

Group A consist from50 patients participate in the study, 6 patients died (12%) (4 during hospitalization and 2 after discharge) and another 2 patients(3%) were missed during follow up period of the study, the remaining was 42 patients. The died patients were those who were in deep coma (6%), who have multiple sites of bleeding on CT of brain (4%) and those who got complications e.g. aspiration pneumonia or bed sores is (2%).

Group B (50)patients included in this study, 12 patients died (24%) 7 during hospitalization and 5 after discharge. The died cases were those who have coma (8%) and those who have multiple sites of bleeding on CT of brain(2%) and those who got complications e.g. aspiration pneumonia or bed sores (6%), while those who have past attack and drugs history were (4%) for each one. The overall incidence fatality rate of both group were 18%. The study show that there is a significant difference in fatality rate between two groups {P value= -1,402(<0.05)S}.

Discussion

This study is used to prevent or decrease the attack of early and late post IPH seizures by using depakine. The results show, 80% of the IPH were above 45 years**,** this finding was approximately similar to the other studies [11] which showed 77.3% were between 50-79 years and this study utilized the same criteria of our study.

Our study represent 14.28% of those received prophylactic depakine developed seizures, this finding relatively agreement with study used same criteria and give prophylactic antiepileptic agent , showed that the frequency of post stroke seizures has been reported from 5-10% in the far West[12]. A slightly higher frequency i.e. 13% is reported from India [13].

Regarding time of fits,4 patient 9.52% (of total 6 patients with fits in group A) developed early seizure less than two weeks in IPH , who treated with depakine, when performed comparison with patients not received depakine in which 8 patients 21.05% (of total 13 patients with fits in this group) associated early seizure less than 2 weeks. Theirs is a significant difference between the two groups.

This result is agreement with study [13] which was show that prophylactic depakine used in IPH was associated with reduced early seizures (<two weeks) to 8.7%, as well as improved neurological deficits at 30 days. Whereas in a retrospective study [14, 15] of patients with IPH, prophylactic used of phenytoin was associated with an independent poor outcome at three months increased incidence of early and late seizures as compared with depakine, the reasons could be due to the high risk for adverse reactions of phenytoin such as sedation, intolerability and high risk of toxicity. moreover ,Phenytoin had a high incidence of cognitive disturbance [16] as compared with depakine and for these reasons present study didn't choice phenytoin.

Other study [17] result show the majority (92% n=90) of post IPH seizures were late type and only 9% were early seizures in cases using depakine.

Our study result show that in group A, 9.52% (4) patient from (6) patient were partial seizure , the other one patient (2.38%) was myoclonic and one (2.38%) had tonic clonic , while in control group patients the types as follows; 18.42% (7 patients) are partial, 7.89% (3 patients) were myoclonic and 7.89% (3 patient) were tonic clonic in type.

Our study result show a better outcome in IPH in states of giving prophylactic depakine regarding post-stroke seizure incidence and consistent results obtained by *Passero et al*.[18] reported that the administration of prophylactic anticonvulsants reduce the risk of acute seizures.

But in certain retrospective study [19] to assess the effect of prophylactic AED on outcomes, found that prophylactic AED use was associated with poor outcome, independent of other established predictors.

The analysis in this difference in two studies result from used type of drug, regarding our study , the drug used is depakine as a AED which is a good popular AED, free of major neurological side effects, low toxicity rates and good tolerability except for pregnancies, while the other study had been used the phenytoin that is known to be full of side effects like CNS depression, drug-induced toxicity especially elderly are highly susceptible to this effect and other effects and there is a study emphasized that phenytoin prophylaxis was also associated with poor outcome [20].

According the fatality rate, our study observed a low fatality rate in IPH patients taking depakine 12% than patients without depakine treatment 24% and the positive effect of prophylactic AED on fatality rate, these finding approximately similar to the study [21] that assessed the fatality rate of IPH in general without giving prophylactic AED, show result that the fatality rate six months after spontaneous intracerebral hemorrhage ranges from 23- 58%.

Conclusion

The non-traumatic non-aneurismal intraparanchymal hemorrhage occurs most commonly between 45-78 years.

The overall incidence of seizure is 23.75%.

The incidence of seizures can be reduced by administration of depakine as a prophylactic anticonvulsant in a patients with IPH.

Partial seizures are more common than other seizure types occurring.

The fatality rate after IPH is 24%.

The use of depakine as prophylaxis drug for IPH fits had a significant role in reducing the fatality rate and in improving survival.

Conflict of Interest: Nil

Source of Funding : Self

Ethical Clearance: Informed consent was obtained from all patient that participate in the study

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Comparison of Prophylactic Effect of (Metoclopramide Plus Dexamethasone) And (Ondansetron) in Female Patients Underwent Laparoscopic Gynecological Surgeries

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Abstract

Post-operative nausea and vomiting were common complicationsin laparoscopic gynecological surgeries. The study was done to evaluate the effectiveness ofondansetron againstcombination of metoclopramide plus dexamethasone in preventionof postoperative nausea and vomiting (PONV) in laparoscopicgynecological surgeries. Fifty ASAI and II consenting patients aged 20–50 years scheduled for laparoscopic gynecological surgeries wererandomlyallocated into two groups ; each group was received either intravenous ondansetron (4 mg) or intravenous metoclopramide (10 mg) plus dexamethasone (8 mg)before inductionof anesthesia. post operativenausea and vomiting then monitoredin period of 4 hours before discharging the patients. Nausea was seen in10 (40%) of patients of ondansetron groups as compared with 16 (64%) in metoclopramide plus dexamethasone .Nausea with blenching developed in 1 (4%) patients of ondansetron group as compared with 8 (32%) patients ofmetoclopramide group. The post operative nausea and vomiting in the ondansetron group was significantly minimum as compared with metoclopramide plus dexamethasone group as p value was 0.01, only 1 (4%) patient of metoclopramide plus dexamethasone group was develops less than 3 times vomiting and non of both groups were develop more than 3 times .Ondansetron was superior to metoclopramide plus dexamethasone in prophylaxis of post operative nausea and vomiting in patients undergoing gynecological laparoscopic surgeries.

Keywords: *Metoclopramide, nausea, ondansetron, vomiting*

Introduction

Postoperative nausea and vomiting, defined as nausea and/or vomiting that occurs within 24 hour after surgery, affects 20%-30% of patients [1][2], the etiology of PONV is thought to be multifactorial, involving individual, anesthetic, and surgical risk factors [3]

Nausea and vomiting are under control of the vomiting center which is found in the medulla. This center takes its stimulations from five primary afferent pathways which include:[4] The chemoreceptor triggering zone , The vagal mucosal pathway in the gastrointestinal tract ,Neuronal pathways from the vestibular system, Reflex afferent pathways from the cerebral cortex c2,3 and Midbrain afferents.

The activation of the vomiting center is done by stimulation of the muscarinic, dopaminergic, histaminergic or serotonergic receptors[5]

The Risk Factors for PONVare either patients , anesthetic or surgical causes; the Patient-specific factor which increase the risk of PONV are female gender [6]; no smoking habit[6]; history of previous PONV or history of motion sickness.[7] while Anesthetic factors aredue to use of volatile anesthetics[8]; nitrous oxide[9]; or postoperative opioids[10].

Surgical factorsincludes type of surgery and duration of surgery, where there is an increase in the PONV in 60 % of patients with each 30minute increase in the duration of the surgery .[7]

Aim of Study

Compare the effectiveness of prophylactic dose of Ondansetron versus (Metoclopramide plus

Dexamethasone)dose in prevention of postoperative nausea and vomiting in female patients undergoing Laparoscopic Gynecological Surgeries.

Material and Method

From September 2017 to September 2018 in the post anesthesia care unit (PACU) and surgical ward at the Baghdad Teaching Hospital, 50 female patients had (American Society of Anesthesiology (ASA) Grade I and II), aged (20-50) years old, weights (65-85)kg were undergoing gynecological laparoscopic surgery (diagnosed and therapeutic) that not exceeded 1hour.

The studied sampleswere divided in to two groups (group A and group B), 25 cases for each group. Group A was received ondansetron (4mg/2ml) I.Vwhile group B was receive dexamethasone (8mg/2ml) plus Metoclopramide (10mg/2ml) I.V.before induction of anesthesia.

Same standard anesthesia technique was used in all cases. Patients were monitored during anesthesia by continued noninvasive blood pressure (NIBP), electrocardiogram (ECG), and peripheral oxygen saturation (SpO2). General anesthesia was induced with Propofol (1.5-2)mg/kg, Ketamine 0.5mg/kg I.V and Rocuronium 0.6mg/kg was given to facilitate tracheal intubation which was done with an appropriate-sized endotracheal tube Midazolam 0.15 mg/kg I.V, and tramadol up to 100mg I.V. was given to patients ,Anesthesia was maintained with (0.7%) (inspired concentration) of halothane in oxygen.

At the end of the surgery, residual neuromuscular block was reversed by neostigmine (0.05 mg/kg) with atropine (0.02 mg/kg) I.V. Extubation was done and after achieving adequate recovery, then patients were transferred to the post-anesthesia care unit (PACU).

The first 4 hours post-operatively nausea and vomiting were assessed by trained nursing staff and the resident doctors, as a routine work in PACU and surgical ward.

The nausea and vomiting were assessed at 0.5 hour ,0.5 hour -1 hour,2 hours ,3 hours and 4 hours . and the intensity of vomiting was assessed by less than 3 times or more than 3 times vomiting

Findings

Table (1): Number and percent of patient with PONV in each group within first 4hr post-operatively.

Time	PONV	Group A	Group B
First 30 mints	Nausea	**1 (4%)**	**2 (8%)**
	Nausea and Belching	0 (0%)	0 (0%)
	Vomiting <3 time	0 (0%)	0 (0%)
	Vomiting >3 time	0 (0%)	0 (0%)
0.5-1hr	Nausea	**5 (20%)**	**8 (32%)**
	Nausea and Belching	**1 (4%)**	**4 (16%)**
	Vomiting <3 time	0 (0%)	0 (0%)
	Vomiting >3 time	0 (0%)	0 (0%)
After 2hrs	Nausea	**4 (16%)**	**5 (20%)**
	Nausea and Belching	0 (0%)	**3 (13%)**
	Vomiting <3 time	0 (0%)	0 (0%)
	Vomiting >3 time	0 (0%)	0 (0%)
After 3hrs	Nausea	0 (0%)	0 (0%)
	Nausea and Belching	0 (0%)	**1 (4%)**
	Vomiting <3 time	0 (0%)	0 (0%)
	Vomiting >3 time	0 (0%)	0 (0%)
After 4hrs	Nausea	0 (0%)	0 (0%)
	Nausea and Belching	0 (0%)	**1 (4%)**
	Vomiting <3 time	0 (0%)	**1 (4%)**
	Vomiting >3 time	0 (0%)	0 (0%)
Total		**11 (44%)**	**25 (100%)**

Group A :ondansetrone I.V.

Group B : Metoclopramide plus dexamethasone I.V.

Table (2) : Numbers and percent of patients with post-operative nausea and vomiting and need of antiemetic drug for each group.

Group	No.of Patient	Post-operative Nausea and Vomiting				
		Null	Nausea	Nausea and Belching	Need of anti-emetic drug Post-op.	No. of patient with complication
Group A	25	14 (56%)	10 (40%)	1 (4%)	0 (0%)	11 (44%)
Group B	25	0 (0%)	16 (64%)	8 (32%)	1 (4%)	25 (100%)
P-Value						**MCP<0.01 (HS)**

Group A :ondansetrone I.V.

Group B : Metoclopramide plus dexamethasone I.V.

Table (3) Numbers and percent of patients that not develop PONVand those needed to anti-emetic drug post-operatively in each group.

Type of Group	Patient without PONV	Need for anti-emetic drug post-op.
Group A	***14 (56%)***	0 (0%)
Group B	0 (0%)	***1 (4%)***

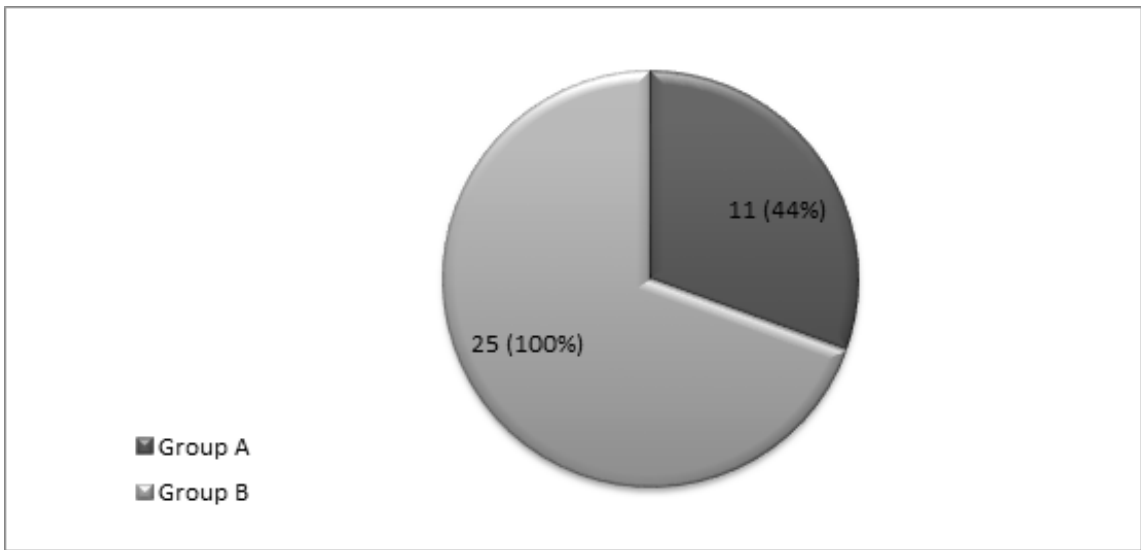
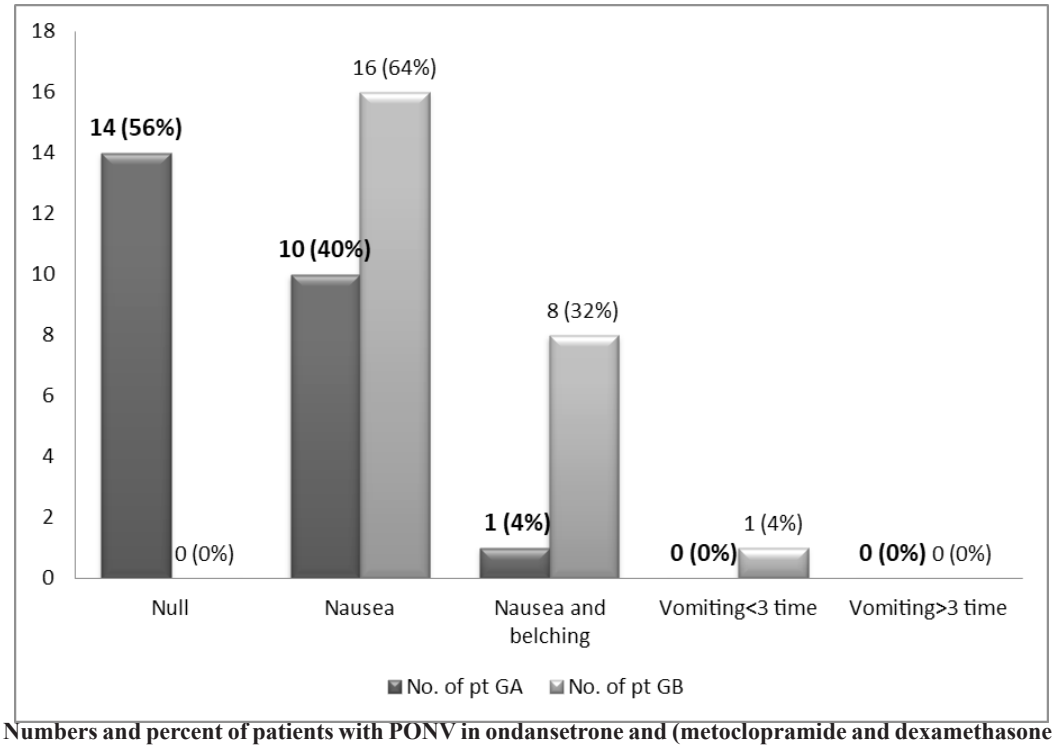


Figure (1): Numbers and percent of patients with PONV in ondansetrone and (metoclopramide and dexamethasone combination)



Figure(2): Numbers and percent of patients with PONV in ondansetrone and (metoclopramide and dexamethasone combination)

Discussion

In this clinical study, there were no significant differences between the two groups regarding the age and duration of surgery. during 4 hours period PONV was significantly minimum in ondansetron group as compared with metoclopramide plus dexamethasone combination group, p value was 0.01ondansetron group 11(44%) patients were developed PONV as compared with metoclopramide plus dexamethasone combination group in which all patients 25 (100%) were developed PONV . This high number of PONV was due to gender of patients (female) ,undergoing laparoscopic surgery and due to uses of volatile and opioid during operation.The patients with no PONV were 16 (56%) in ondansetron group while all patient with metoclopramide plus dexamethasone group had PONV.Patients with nausea only develops in 10 (40%) in ondansetron groups as compared with 16 (64%) in metoclopramide with dexamethasone combination .Nausea and blenching were developed in 1 (4%) patient in ondansetron group as compared with 8 (32%) in metoclopramide with dexamethasone combination group.The majority of patients were develop nausea with or without blenching in both groups , Only 1 patient (4%) develop vomiting after 4 hour of extubation of less than 3 times and treated with antiemetic .

The other studies that were compatible with our study :Tabari M et al.(Iran ,2014) in which they compare the ondansetron , metoclopramide and dexamethasone and shows that the ondansetron had the minimum incidances of PONV as compared with other groups [11]. When compare the Ondansetron with metoclopramide ,granisetron and dexamethasone and they showed that the Ondansetron had the minimum incidances of PONV as compared with metoclopramide but the granisetron had minimum as compared with ondansetron[12]. In Nigeria study conclude that ondansetron was more effective than metoclopramide in the prevention of nausea in patients undergoing elective day−case gynaecologicallaparoscopic procedures. Both metoclopramide and ondansetron were effective in the prevention of vomiting but ondansetron still offered superior prophylaxis. There were no significant side effects for both metoclopramide and ondansetron when used as prophylaxis.[13]. So results of Waqas *etal.,* they conclude that Ondansetron was more effective as compared to combined metoclopramide and dexamethasone, in the prevention of post-laparoscopic surgery nausea and vomiting[14]

Conclusion

We conclude that the ondansetron had better prophylactic antiemetic action than metoclopramide

Conflict of Interest: Nil

Source of Funding : Self

Ethical Clearance: Informed consent was obtained from all patient that participate in the study

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Effect of Silver Nano-Fillers and Aging on Tensile Strength of Heat Cured Acrylic

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Abstract

Background: Silver nanoparticles are attracting interest as antibacterial and antimicrobial agents as incorporating materials into PMMA denture resin, but the need for application without side effects on resin properties is essential.This study was oriented to investigate the addition of silver nano-fillers(AgNPs)and aging on tensile strength of acrylic resin polymerized by two techniques water bath and autoclave.

Materials and method: 120specimens made of heat-cured acrylic were divided into two main groups depending on curing methods water-bath and autoclave processing technique, and then each group was subdivided into experimental groups according to the addition of silver nano-fillers in two concentration(0.2% and 2%AgNPs)and control groups(0%AgNPs). Each subgroup was divided into 2groups according to the period of immersion in distilled water(1day and 1month)with(N= 10).The WDW200Esteel tensile strength tester was used for measuring the tensile strength for all groups.Data were statistically analyzed using One-way ANOVA-test and Independent t-test to detect the significant different among tested and control groups at significance level ($P \leq 0.05$).

Results: A highly significant decrease in tensile strength was observed with the addition of silver nanofillers to PMMA.According to curing methods, the results showed that there wasnon-significant difference of curing in water-bath and autoclave technique. In addition, by aging specimens for one month period, there was a highly significant decreasein the tensile strength of acrylic in all groups .

Conclusions: The addition of AgNPs to acrylic lead to reduction of its tensile strength.No change in the tensile strength by using autoclave method for acryliccuring. In addition, the prolong storage period was reduced tensile strength of the resin.

Keywords: *AgNPs, heat-cured acrylic, aging, autoclave, tensile strength.*

Introduction

The poly methyl-methacrylate was introduced by Wright in1937, from this period it considered as the main polymer for fabrication of denture base[1]. It is well-known that acrylic resins may act as a reservoir for microorganisms and contribute to re-infection in denture users;there was a demand for effective widely-spectrum antimicrobial resin materials in dentistry[2]. Therefore, more attention has been directed towards the AgNPs as incorporated particles into acrylic to improve its characteristics. The silver in nano-form is used to avoid the contamination or to make better various physical and mechanical properties of denture[3]. There are lot of researches on introducing nano-silver to acrylic. One of these studies allowed to obtain materials which show increased antifungal effectiveness in vitro tests[4],and relevant basic mechanical properties[5]. Hamedi-Radetal.,2014compared the conductive thermal,compressive strength, and tensile strength of the resinthat reinforced with nano-silver[6].A research has revealed the incorporation of AgNPs to the polymer powder was higher in thermal conductivity than that of the conventional polymer[7].Other study investigated there was improvement in viscoelastic properties of acrylic denture reinforced with AgNPs[8].Ghaaffari

and Hamadi Rad,2015studied the effect ofAgNPs on the tensile strength of PMMA[9].Furthermore, over the years, curing procedures have been modified with a view to improve the physical and mechanical properties of resin materials, the water bath processing technique has been the most conventional polymerization technique[10]. In spite of the advantages provided by this technique like the ease, simplicity and cost-effectiveness, a major disadvantage has been long processing time need[11]. The use of pressure cooker for denture polymerization was first reported in1976.The investigation by Indian researchers of the pressure cooker for curing resin was done and they revealed that itrequires less than 1hour for curing and utilizes conventional equipment[12].

On the other hands,variousstudies in which distilled water was used for aging[13,14].The flexural strength and bond strength of denture base and relined resins wereobserved after water immersion[15,16].AlsoJagger etal.,2000 studied the effect of 6months of storage in water at 37°C on heat polymerizing acrylic[17].

So this study was aimed to evaluate the effect of:

1.Incorporating 2% and 0.2%AgNPs into heat-cured acrylic denture-based material on the tensile strength of resins.

2.Two processing techniques: water-bath and autoclave on the tensile strength of heat-cured resins.

3.Immersion in distilled water in two periods(1day and 1month)on the tensile strength of heat-cured resins.

Materials and Method

A total of 120 specimens were prepared from heat-cured denture base resin,(Superacryl`plus, Modern Medical equipment, Czech Republic) which were divided into two groups according to processing technique:water bath and autoclave. Each onedivided into three groups according to AgNPs concentration:0%AgNPs,0.2%AgNPs,and2%AgNPs. The samples for each group were subdivided depending on the period of immersion in distilled waterfor 1day and 30days(N=10).The AgNPs(Silver Nano-powderproduct no.MKN-Ag-090,MK Nano,MKImpexCorp.,Lot no. 0729,Canada),silvernano-powderof 90nm particle size according to manufacturer instruction(Fig.1).

Sample preparation

The wax patterns(Polywax, Bilkim chemical company, Turkey)were constructed in form ofFlat dumbbell-shaped with dimensions**(80mm,9mm,3mm)** length,width,thickness respectively[18](Fig.2).

The mould preparation was utilized by placing the wax pattern after coating with separating medium in the lower portion of the flask within dental stone typeIV(Elite stone, Zhermack, Germany)that was mixed according to the manufacturer's instructions.The wax patterns were placed about 1/2 of their thickness. After that,the opening of the flask was carefully done and the patterns were removed from the mould**.**

Incorporation of silver nanoparticles:

For experimental specimens,AgNPs were added in two percentages(0.2%,2%) to the monomer through the extremely sonication of the fillers which had been well dispersed in the liquid by a probe of sonication equipment (Soniprep150, England)at 120W and 60KHzto split them into nano-crystals for 3minutes individually[19]. The monomer with AgNPs were mixed with acrylic powder immediately to reduce the possibility of particle aggregation and separation.

The proportion of acrylic mixing was 10g: 4.4ml(P/L)according to the manufacturer's instruction and left to stand until a dough stage was reached. An electronic balance with 0.0001g accuracy (StaroiusBP 30155, Germany)was used to measure the weight of materials of this study.

Packing and Curing

The dough stage of the mixture was packed in the mould after lining with a separating medium and become ready for curing by **water-bath technique**,the curing was done byplacing theconventional brass metal flask (Broden,Sweden)in water bath (Memmert, Germany) and processed by heating at 74°C for an hour and a half.The temperature was increased to the boiling point for 30 minutes[20].Then, the metal flask was allowed to cool at room temperature for 30minutes, followed by complete cooling of the metal flask for 15 minutes before deflasking. For curing by **autoclave processing technique**,it was carried out by placing the clamped flask in a fully automatic autoclave(QD, England)and processed by the preprogrammed cycles (121ºC/210KPa,30min). Before placing the clamped flask inside the autoclave, the autoclave must be leveled and filled with distilled water. Then, the clamped flask

placed in the tray and pushed inside the chamber, then closed and secured the door. The autoclave operated and started heating the water, then the temperature and pressure were raised till its reached(121ºC and 210KPa) respectively. When the temperature reached(121ºC), temperature and pressure held automatically at(121ºC and 210KPa) respectively for 30min[21], then automatically exhausted the steam and the programmed cycle was finished, then removed the clamped flask.Then the flask was opened and the specimens were removed from the stone mould, smoothing and polishing in conventional way with continuously cooled with water to avoid overheating which may lead to the distortion of the specimens[20].

Half of the specimenswere soaked indistilled waterfor 1day.While other half immersed in distilled water 30days in incubator(Gallenbamp, England) at 37±1ºC and the water changed daily within the mentioned period[13].

Testing Procedures

The acrylic specimens were utilized to measure the tensile strength(Fig.3),that was achieved by using(**WDW200E steel tensile strength tester with tensile strength testing machine)(**Fig.4). Each specimen was positioned on bending fixture, consisting of 2 parallel supports(50mm)apart, the full scale load was(50g),and the load applied with cross head speed 5mm/min by rod placed centrally making deflection until fracture occurred and the force were recorded in Newton.

The tensile strength values were calculated by following equation:

Tensile strength=F/A[18].

F **=force at failure(Newton).**

A**=Minimum cross sectional area(mm).**

Data was analyzed by Statistical Package for Social Science (SPSS) version #21(SPSS, Chicago, Illinois, USA).Descriptive statisticsand inferential statistics: One-way ANOVA-test and Independent t-test were used to detect significant differences between tested and control groups at a significance level($P \leq 0.05$).

Results

Descriptive statistics of tensile strength values of the groups without the addition of AgNPs group showed higher mean values than the experimental groups with addition of 0.2%, 2% AgNPs.The tensile strength for those ofone day immersion in distilled water showed higher mean values thanthose immersed for thirty days (Table1, Fig.5).

For comparing the effect of silver nano-filler addition on the tensile strength of the heat cured acrylic, One-way ANOVA-test showed there was a highly significant different between the tested groups(Table2). Further analysis by LSD-test was showed there was a highly significant different between the control group and experimental groups(Table3).

In comparison, the effect of curing technique the Independent t-test was showed there were non-significant differences in the tensile strength between the water bath groups and autoclave groups**(**Table4**).**

Furthermore, the means values of the tensile strength of the acrylic resin depending on the time of immersion in distilled water were compared using the Independent t-test which showed that there were highly significant differences among the study groups(Table5)**.**

Table1: Descriptive statistics of tensile strength(N/mm²)for all study groups.

Study Groups			N	Mean	SD	Minimum	Maximum
Water bath	0%AgNPs	1day	10	48.112	2.5578	42.325	50.567
		30days	10	44.365	1.2853	42.520	45.932
	0.2%AgNPs	1day	10	45.820	1.2958	43.976	47.356
		30days	10	41.928	1.3539	40.257	44.050
	2%AgNPs	1day	10	42.722	2.8872	37.268	45.907
		30days	10	39.722	1.5263	35.910	41.181
Autoclave	0%AgNPs	1day	10	46.297	1.1601	44.358	47.184
		30days	10	43.122	1.5407	39.520	44.955
	0.2%AgNPs	1day	10	44.728	1.0435	43.069	46.499
		30days	10	40.981	0.8876	39.296	42.266
	2%AgNPs	1day	10	40.875	1.2130	38.065	42.156
		30days	10	38.638	0.8136	37.332	40.013

Fig. 5:Bar chart showed tensile strength test for all groups.

Table 2:O-way ANOVA-Test comarison the tensile strength between groups according to addition of different concentration of AgNPs.

			F-test	P-value	Sig
Water bath	1day	0%AgNPs&0.2%AgNPs&2%AgNPs	13.257	0.000	HS
	30days	0%AgNPs&0.2%AgNPs&2%AgNPs	27.827	0.000	HS
Autoclave	1day	0%AgNPs&0.2%AgNPs&2%AgNPs	59.448	0.000	HS
	30days	0%AgNPs&0.2%AgNPs&2%AgNPs	39.451	0.000	HS

HS: highly significant at P≤0.01.

Table 3: LSD-test for comparison between thegroups according to addition of AgNPs.

			P-value	Sig
Water bath	1day	0%AgNPs&0.2%AgNPs	0.038	S
		0%AgNPs&2%AgNPs	0.000	HS
		0.2%AgNPs&2%AgNPs	0.007	HS
	30days	0%AgNPs&0.2%AgNPs	0.001	HS
		0%AgNPs&2%AgNPs	0.000	HS
		0.2%AgNPs&2%AgNPs	0.001	HS
Autoclave	1day	0%AgNPs&0.2%AgNPs	0.005	HS
		0%AgNPs&2%AgNPs	0.000	HS
		0.2%AgNPs&2%AgNPs	0.000	HS
	30days	0%AgNPs&0.2%AgNPs	0.000	HS
		0%AgNPs&2%AgNPs	0.000	HS
		0.2%AgNPs&2%AgNPs	0.000	HS

S:significant at P≤0.05,HS:highly significant at P≤0.01

Table 4:Independent T-Test for compariosn the tensile strength between groups according to curing techniques.

			t-test	P-value	Sig.
0%AgNPs	1day	Water bath&Autoclave	2.064	0.054	NS
	30days	Water bath&Autoclave	1.960	0.066	NS
0.2%AgNPs	1day	Water bath&Autoclave	2.076	0.052	NS
	30days	Water bath&Autoclave	1.850	0.081	NS
2%AgNPs	1day	Water bath&Autoclave	1.865	0.079	NS
	30days	Water bath&Autoclave	1.981	0.063	NS

NS:non-significant at P>0.05

Table 5: Independent T-Test for comparsion the tensile strength between groups according to storagetimein distilled water.

			t-test	P-value	Sig
0%AgNPs	Water bath	1day&30days	4.139	0.001	HS
	Autoclave	1day&30days	5.177	0.000	HS
0.2%AgNPs	Water bath	1day&30days	6.567	0.000	HS
	Autoclave	1day&30days	8.648	0.000	HS
2%AgNPs	Water bath	1day&30days	2.905	0.009	HS
	Autoclave	1day&30days	4.843	0.000	HS

Discussion

Many studies pointed onAgNPseffect when added into acrylic for the treatmentof oral infections like denture stomatitis and other antifungal and antibacterial infections. So, the modification of denture resins by silver additive in nano-form to get the possible positive influence of its addition[3].Silver nanofillers are smaller in size andthey possess physical, chemical, and biological properties that are distinctive from those presented by traditional bulk materials. Finer size and larger surface area provide potent antibacterial impact at a low filler level, so determining AgNPs concentration necessary for its efficacy and avoiding negative effect on mechanical properties[22],so the decision was made to choose two percentages of AgNPs about 0.2% and 2% depending on the previous study that was revealedthe addition of different percentageof silver nanoparticles to acrylic resinabout 0.2 and 2% result in increasein the compressive strength of acrylic[9],other research conducted that the use of 0.2%,2% nano-silver with acrylic resin increases its thermal conductivity[7],the study was showedthere was decrease in tension properties as a result to addition of5%AgNPs[9].Furthermore, increase the percentage ofsilver nanoparticlesthat leads to adverse effect on properties of resin such as the addition ofsilver nano-filler lead to color change of acrylic because the fillers tend to fill any spaceswithin the polymer,and decrease the amount of transmitted light[23].As well as silver nano-fillers are metal fillers which are changing thecolor of acrylic resin that limits its application inthe esthetic zone that was increase with the use of more amount of nano-filler[7].

The result of effect of silver addition in nano-formon the tensile strength was decrease in this properties when compared with no addition, this was probably attributed to AgNPs acts as impurities and tensile strength would decrease by increasing their content.Anotherexplanation was the reductionof monomers reaction and increases the amount of unreacted monomer, behaving like a plasticizer[24].The plasticization effect of the resultant residual monomer will reduce the molecular binding force, that lead tomultiple micro-fracture in high

deformation upon stretching that weaken the resin[2]. In addition, there was an increase in impurity action of nanofillers which cause a stress concentrated points in the matrix and weakening the resin[25].This result in the brittleness of resin may be attributed to the dysfunctional effect of high percent of AgNPs because the low absorbed energy ion within fracture and Ag^{+}ions being reduced as the concentration of silver increase that was generating atom clusters and smaller particle size during the polymerization process[26]**.**These findings are consistent with previous studies who reported that the mechanical and physical properties of the composite are influenced by silver nanofiller concentration since mechanical properties of composites decreased by increasing silver nanoparticles[27,4].

According to the result of this study, there was no difference between the curing in water bath and autoclave curing. This result in agreement with the findings of previous study that showed that there was non-significant difference between the autoclave and water-bath curing regarding tensile strength[28].This could be explained that "autoclave" utilized the temperature and pressure when water is heated in a sealed vessel such as an autoclaveand the pressure rises due to the constant volume of the container. The boiling point of water is then raised because the amount of energy needed to formssteam against the higher pressure is increased[29,30]. Another cause is high heat treating,could be attributed to increase in cross-linking that provides a sufficient number of bridges between linear macromolecules to form three-dimensional network that decreases water sorption, decreases solubility, and increases the strength and rigidity of resin, as well as the most important determinant of resin strength is the degree of polymerization exhibited by the material.As the degree of the polymerization increase, the strength of the resin also increases, the polymerization cycle employed with a heat activated resin[31].

On the other hand, the effect of the aging in distilled water on the tensile strength of the acrylicwas showed the decrease with increase the period of exposure,this result confined with the study that found the prolonged storage in water badly effecton acrylic resin[17], another research showed the aging in water or aqueoussolutions lead to decrease the mechanicalproperties of the resin, they explainedthe reasons by the use of water may affect the mechanical properties of the acrylicand cause a reduction in the denture strength. This could be attributed to the plasticizer, the materials become softer and tougher, with lower tensile strength[13],this may be due to the gap between polymer chains that wascaused distilled water penetration and resulted the change in the mechanical properties of the polymer[32].

Conclusion

There was weakening in the tensile strength of resin due to 0.2% and 2% silver addition in nano-form. On the other hand, the autoclave polymerization technique can be used as alternative method for processing denture resins, but the prolong storagein water decreased the tensile strength.

Conflict of Interest: non

Source of Finding: self

Ethical Clearness: non

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Formulation and Evaluation of Acetic Acid Lotion for the Treatment of Wound Infection

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Abstract

Acetic acid has been used as a topical agent for healing wound infections. However, no studies have been done to formulate acetic acid based lotion and investigate its role in wound dressing. Therefore, the current workinvolved study the antibacterial activity of acetic acid against pathogenic bacteria associated with wound infections in vitro. In addition to formulate acetic acid lotion and study its role in the treatment of infected wounds, using mice model.

The results show that acetic acid has antibacterial efficacy at different concentrations against pathogenic bacteria tested; *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter* sp, *Proteus*sp and *Salmonella* sp.

The application of acetic acid based lotion on wound infected with *P. aeruginosa*twice a day for 6 days leads to prevent bacterial infection and treat wounds.Herein, we reveal that acetic acid lotion has potent antibacterialefficacy and plays a significant role in wound healing.

Key words*: Wound, bacterial infection, Acetic acid, wound healing.*

Introduction

Wound infection is a main problem contributing to mortality and morbidity due to the rise in the infections that are caused by multidrug resistant bacteria.To overcome this problem, variety alternatives have been suggested such as bacteriophages, vaccines, antimicrobial peptides, and plant extracts, etc. [1].Organic acids, such as acetic acids, are promising alternatives for infection inhibition [2].

Acetic acid has been used in medicine for thousands years.Many studies address antimicrobial activity of acetic acid against a wide range of pathogens.Acetic acid has been used to inhibit bacterial biofilms of both Gram negative and Gram positive bacteria such as *P. aeruginosa*, *K. pneumoniae*and *S. aureus* [3].In addition, it has been shown that using 6% acetic acid leads to reduce *Mycobacterium tuberculosis* after 30 min of exposure [4].

In literature, Acidic acid was used as a topical agent for healing war-wound infections caused by *P. aeruginosa* [5]. It has been reported that treating burn wounds with 5% acetic acid result in inhibit *P. aeruginosa* infection [6].

In addition to antibacterial activity, acetic acid has anti-fungal activity against pathogenic fungi. Recently, it has been found that low concentrations of acetic acid were active against Mucorales species [7].

Antimicrobial activity of acetic acid is attributed to low pH. However, it has been found that using another acids such as HCl does not show same antimicrobial effect as acetic acid even when they wereused at the same pH. Thus, the antimicrobial activity of acetic acid could be result of the acetic acid molecule itself [3].

To date, no study has been done to formulate acetic acid lotionand investigate its role in wound healing.Acetic acid is available, cheap, non-toxic and has antimicrobial activity. We propose suggest that initial application of Acetic acid lotion may represent an effective way to impede bacterial growth, and thus inhibit wound infection.

Materials and Method

Bacterial strains& Media

Pathogens cause wound infections such as

S. aureus, *E. coli*, *P. aeruginosa*, *Acinetobacter* sp, *Proteus*sp and *Salmonella* sp. were diagnosed and kindly provided by clinical laboratories at Al- Yarmouk hospital.*S. aureus* was grown on mannitol salt agar plates. Gram negative pathogens were grown on MacConkey agar plates at 37 °C. Agar well diffusion experiments were performed in Muller- Hinton agar.

In vitro, antibacterial effects of acetic acid.

Agar well diffusion method was used to determine antibacterial activity of the acetic acid. Acetic acid was diluted in sterile distilled water to prepare the following concentrations 5%, 4%, 3%, 2% and 1% (V/V).

Muller-Hinton agar was inoculated with one of the following bacteria (10^6 cfu/ml, adjusted to 0.5Mcfarlandstandard):*S. aureus*, *E. coli*, *P. aeruginosa*, *Acinetobacter* sp, *Proteus*sp and *Salmonella* sp. 100µl from each concentration was added into 6mm diameter wells. Amoxcillin- (30µg/disc) was used as a positive control. Plates were incubated at 37 ºC for 24 hours.

Formulation of acetic acid based lotion

Table (1) materials used in acetic acid lotion formula

Ingredients	Quantity per 100ml
Acetic acid	5 mL
Mineral oil	10 gm
Tween80+span 60	4 gm
Propylene glycol	5 ml
Xanthan gum	0.5 gm
Methyl paraben	0.03 gm
Propyl paraben	0.02 gm
triethanolamine	Q.S
Perfume	Sufficient quantity
Distilled water	Q.S to 100ml

Q.S: quantity sufficient

Preperation of acetic acid lotion

Oil in water emulsion based lotion was formulated .the emulsifier (span60) and other oil soluble components (mineral oil) dissolved together as oil phase and heated to 75 ºC. the prservatives (methyl paraben and propyl paraben and other water soluble(acetic acid, tween80, propylene glycol) dissolved in aqueous phase and heated to 75 ºC, after that aqueous phase added to oil phase in portions with continuous stirring until the lotion formed then put aside until cool. Xanthan gum prepared as asolution by adding 0.5 gm per 100ml then added to formed lotion with continuous stirring to give suitable consistency. triethanolamin added in drops to adjust the lotion pH at 5.5 which mimic to skin Ph and not cause any irritant [8].

Characterization of acetic acid lotion formula:

Visible inspection

Each emulsion was evaluated to detect visible modifications or instabilities such as color, creaming, coalescence, and/or separation of phases.

In vitro characterization of lotion formulation

1- Viscosity: measurement of viscosity was conducted using a Model Brookfield viscometer. A C-61 spindle was employed with a rotation rate of 2.5, 5, 6 rotation per minute (rpm). Temperature was set at 25°C ± 2 and these experiments were conducted in triplicate 2-detrmination of pH: Lotion pH was recorded with a digital pH meter (Mettler& Toledo, Giessen, Germany) by inserting probe into the lotion formulation and allowing it to equilibrate for 1 minute[9] [10].

The viscositiy was measured to determine rheological properties of formulations.

Brookfield Rheometer viscometer at 30˚C with a CPE 61 spindle at 30 rpm was used to

serve this purpose. Results were taken in triplicate and the average was taken in to

consideration.[1]

The viscositiy was measured to determine rheological properties of formulations.

Brookfield Rheometer viscometer at 30˚C with a CPE 61 spindle at 30 rpm was used to

serve this purpose. Results were taken in triplicate and the average was taken in to consideration.

Technique of application

6 mice (age 8 weeks, weight 30–35 g) were randomly divided into two groups. Group forAcetic acid lotion applicationand group as a control (no treatment). Mice were individually put in clean cages. Following shaving, a skin wound (10 mm x 10 mm) was excised from the dorsum part of mice. Wounds were infected with*P. aeruginosa* (10^6 cfu / ml).The application of acetic acid lotion was started after two hours of infection. Wounds treated with lotion twice a day. The experiment was repeated twice.

Statistical Analysis

Statistical analysis of values was performed with Graph Pad Prism version 5. Unpaired two-tailed t-test was used for statistical significance.

Data were stated as the mean ± SD. *$p < 0.05$.

Findings

Acetic acid displays antibacterial activity against the tested pathogenic bacteria.

In the current work, antibacterial effect of acetic acid was investigated on *S. aureus*, *E. coli*, *P. aeruginosa*, *Acinetobacter* sp, *Proteus*sp and *Salmonella* sp. Agar well diffusion method was used for detecting antibacterial potency. Antibacterial activity was evaluated by measuring an inhibition zone around the well containing acetic acid. Different concentrations of acetic acid (5%, 4%, 3%, 2% and 1%) (v/v) were used.

The results revealed that acetic acid has antibacterial activity against all tested bacteria. All concentrations exhibited antibacterial activity against *Acinetobacter*sp and *Salmonella* sp. The inhibitory effect shown for *Acinetobacter*sp by 5%, 4%, 3%, 2% and 1%) (v/v) was 36, 28,25, 23, and 19 mm, respectively. And the mean of inhibition zone recorded for *Salmonella*sp was 28, 26, 25, 18 and 10 mm, respectively. Acetic acid inhibits *E.coli*, *P. aeruginosa*, *S. aureus* growth at concentrations of 5%, 4%, 3%, and 2%. The mean of inhibition zone revealed for *E.coli* was 29, 28, 27 and 25mm, respectively. And the records for *P. aeruginosa* were 24, 21, 18, and 17mm, respectively. *S. aureus* was also sensitive to these concentrations. The mean of inhibition zones was 25, 20, 17 and 13 mm, respectively.

Only 5% and 4% exhibited antibacterial activity against *Proteus* sp. with mean of inhibition zone of 22 mm and 20 mm respectively. Amoxicillin –clavulate (30µg/ disc) inhibits *E.coli* and *Acinetobacter* sp. growth with mean of inhibition zone of 10 and 12 mm, respectively. Whereas, *Salmonella*sp, *Proteus*sp, *P.aeruginsa* and *S. aureus* shown resistance to this antibiotic (Table 2) & (Figure 1).

Table 2: Antibacterial activity of acetic acid against bacteria related to wound infections

Pathogenic bacteria	**Acetic acid concentrations(v/v) Inhibition zone mean (mm)±SD**					**Amoxcillin-clavulate 30µg/ disc**
	5%	4%	3%	2%	1%	
E. coli	29±1	28±1	27±1.52	25±1	0	10±0.57
Acinetobacter sp	36±1.53	28±1	25±1	23±1.15	19±1.52	12±0.58
Salmonella sp	28±1	26±0.57	25±1	18±1.15	10±1.32	R
Proteus sp	22±0.57	20±0.76	0	0	0	R
P. aeruginosa	24±0.58	21±0.57	18±0.57	17±1	0	R
S. aureus	25±0.57	20±0.28	17±0.57	13±1	0	R

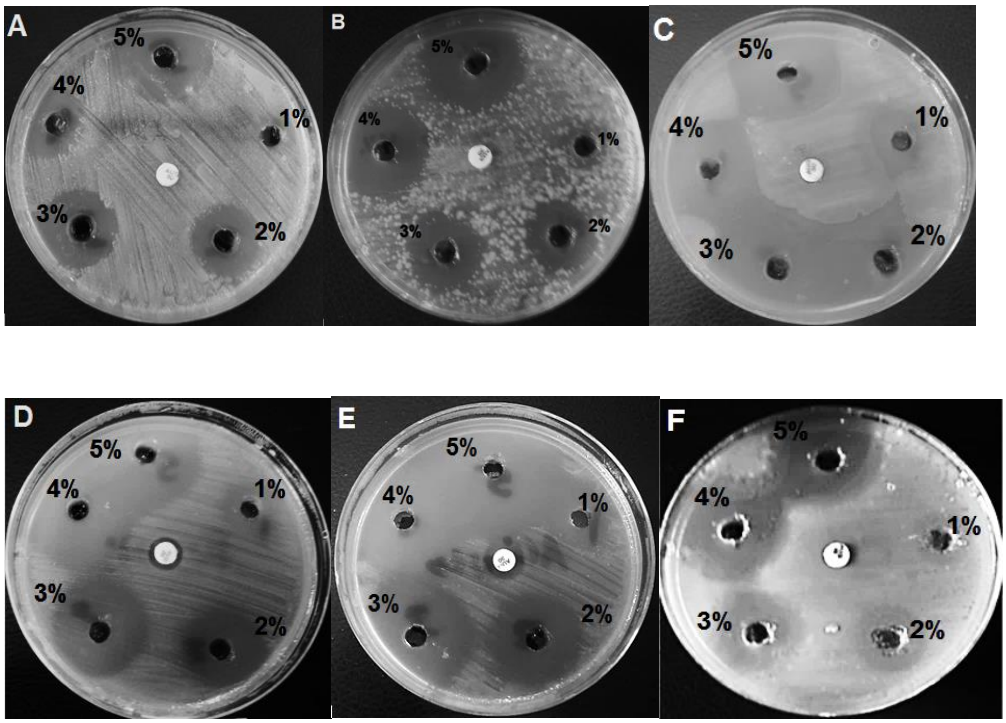

Figure (1): Antibacterial efficacy of Acetic acid.

The figure shows antibacterial activity of acetic acid against (A):*S. aureus,* (B):*P. aeruginosa*,(C):*Salmonella* sp.,(D):*E.coli*,(E): *Acinetobacter* sp. and (F): *Proteus* sp.

Application of acetic acid lotion twice a day for six days leads to prevent *P. aeruginosa* infection as well as wound healing. The results show marked differences between the treated mice and the control (no acetic acid lotion application) (Figure 2). Significant differences between the treated wound size and untreated wounds (Figure 3).

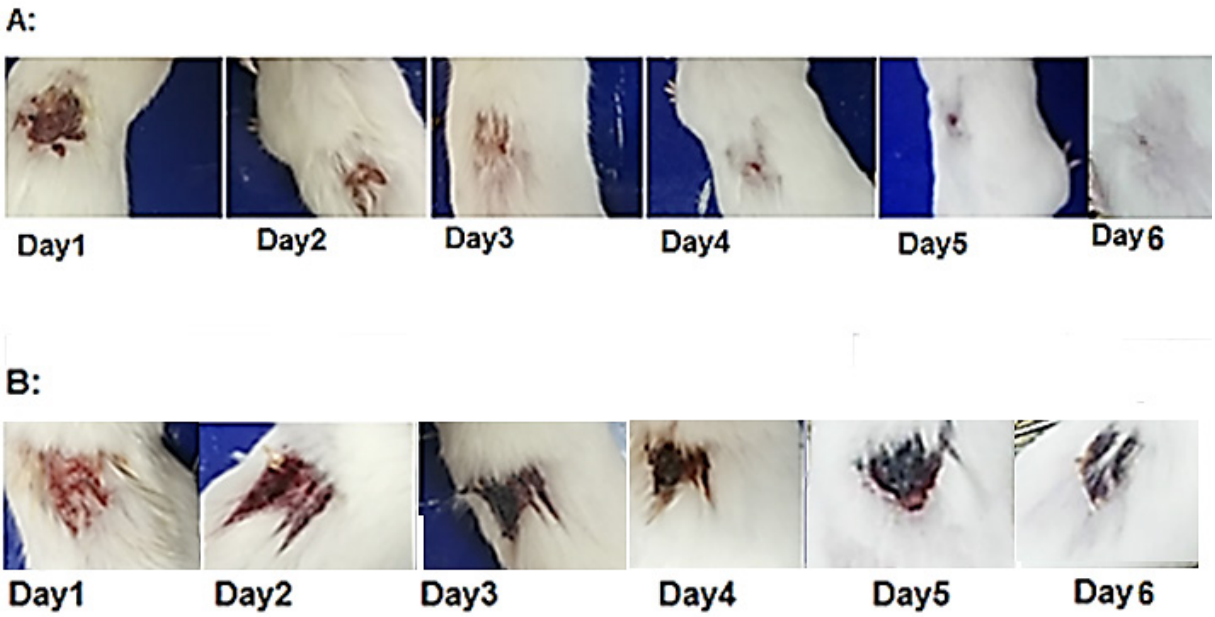

Figure2: Acetic acid based lotion for wound healing.

: Acetic acid lotion application on wound infected with *P. aeruginosa*.

: Control mice infected with *P. aeruginosa.*

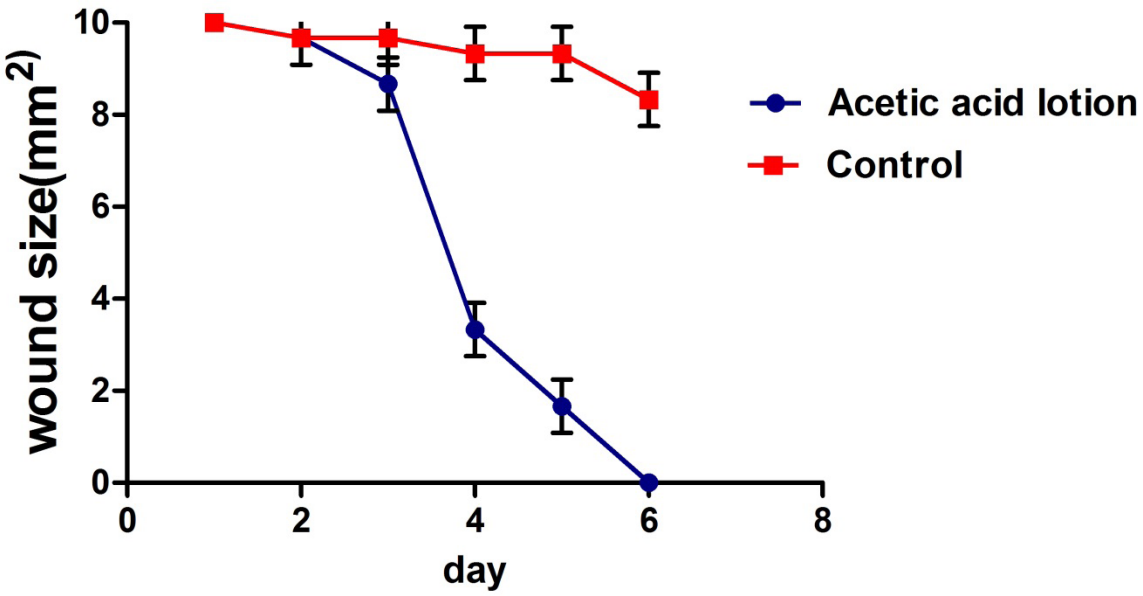

Figure 3: The treatment with Acetic acid lotion enhances wound healing.

Discussion

In the current study, the work involved using acetic acid to inhibit bacterial wound infection and formulate an acetic acid lotion to heal wounds. Pathogens infections are a key problem in the curative of chronic wounds. Usually, antibiotics have been used to treat wound infections, but in the majority of chronic wounds, these antibiotics are un useful. In addition to the cost and the resistance to antibiotics.,Acetic acid is available, cheap, non-toxic and has antimicrobial activity. Thus, we chose it to formulate acetic acid lotion and investigate its ability for wound dressing. In the current study the following pathogenic bacteria that cause wound infections were used: *S. aureus*, *E. coli*, *P. aeruginosa*, *Acinetobacter* sp, *Proteus*sp and *Salmonella* sp.Herein, different concentrations of acetic acid were used to inhibitbacterial growth.

In the current study, we found that acetic acid is effective againstall tested bacteria. Acetic acid demonstrated antibacterial activity against all pathogens. Many studies demonstrate the antibacterial activity of Acetic acid. Anti- bacterial activity of different concentrations of acetic acid (0.5—2%) was investigated against *P. aeruginosa*. The results revealed that 2% acetic acid exhibited the highest inhibition zone [11]. Acetic acid inhibits *E. coli* growth [14]**.** This inhibition was due to diffusion ofthe acetic acid molecule leads to lowering the cytoplasmic pH[15]. The pH is one of the crucial factors that affect bacterial growth.

Next step in our study was formulating acetic acid based lotion (5% acetic acid). The prepared oil in water emulsion based lotion was milky color, acceptable odor, homogenized and stable. The viscosity of acetic acid lotion measured in different rotation per minute (R.P.M) was 1344 centipoise at 2.5R.P.M, 1081centipoise at 5R.P.M and 938centipoise at 6R.P.M so it behave as psudoplastic and has viscosity within normal range. The pH of prepared lotion was 5.5.

To evaluate the role of Acetic acid lotion in wound dressing, acetic acid lotion was applied directly to the infected wounded skin. Wounds were infected *P. aeruginosa*. *P. aeruginosa* is the most frequent Gram negative pathogen that causes chronic wound infections [12]. The results show that the application of acetic acid lotion into the infected wounded skin twice daily for 6 days result in wound healing. 5% acetic acid is a harsh environment for bacterial growth. Thus, the lotion prevents bacterial growth. It has been shown that application of 1% acetic acid for 10-14 days in burn wounds leads to elimination *P. aeruginosa* infection [13].

It is well known that lowering the pH of the wound leads to wound healing. Low pH result in inhibit bacterial protease and decrease bacterial toxicity. In addition, low pH induces host macrophages activity. The pH of the formulated acetic acid lotion in the current work was adjusted to 5.5. This pH does not cause skin irritation since it is close to the pH of skin. In addition, adding Perfume leads to disappear cetic acid smell that makes it acceptable. Antibiotics resistant bacteria are a critical problem in wound healing process. Acetic acid lotion accelerates the treatment of the infected wounds .Thus, no needs to use antibiotics, especially with multidrug resistant bacteria. Since Acetic acid lotion has potential role for wound dressing. We suggest that the application of acetic acid lotion on infected wounds could be a good way to inhibit infections and induce wound healing.

Conclusion

Acetic acid lotion has potent antibacterial efficacy and accelerates the treatment of the infected wounds. Thus, we suggest that initial application of this lotion may represent an effective way to treat wounds especially with multidrug resistant bacteria.

Conflict of Interest: None.

Funding-self or other source: Al mustansiriyah university**.**

Ethical Clearance: all studies were conducted in accordance with the Ethical Committee of College of pharmacy/Al-Mustansiriyah University.

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Evaluation the Effects of Incorporation of Silver Nitrate on Some Mechanical Properties of Soft Liner Materials

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Abstract

Background:- Soft liners placed in dentures have been used as carrier for antifungal drugs in treating denture stomatitis.The aim of this study is to evaluate the effect of incorporated of silver nitrate in different volume into acrylic soft denture liner and to assessment the effect of these additions on some mechanical properties.

Materials and methods: One hundred and twenty (120) specimens were prepared from heat cure soft liner material and divided into three groups according to the test that carried out. Forty specimens for each (hardnesstest, compressive strength test, water sorption and solubility test) . Then each test subdivided into four subgroups according to the volume of the silver nitrate solutionincorporation. Controlgroup which have ten specimens without incorporation of silver nitrate and experimental groups which have thirty specimens with incorporation of(0.05ml ,0.1 ml and 0.2 ml) of silver nitrate solution. All specimens were constructed as in conventional method then stored in distilled water for seven days be ready for measurement.

Findings:- The results of this study showed that highly significant increase in the hardness tested groups also compressive strength group at 0.1 ml of silver nitrate incorporation while significant decrease in 0.05 and 0.2 ml groups.Watersorption and solubility showed that highly significant increase in all volumes of silver nitrate incorporation groups.

Conclusions:- Silver nitrate incorporation was significantly increases the hardness of soft lining material while Compressive strength was significantlyincreased after incorporation of 0.1 ml of silver nitrate .The incorporation of 0.05 and 0.2 ml of silver nitrate to soft liner material significantly was decreased. Regarding water sorption and solubility were significantly increased after incorporationof silver nitrate to soft lining material in all experimental groups.

Keyword*: silver nitrate, soft lining material, hardness, water sorption and solubility*

Introduction

Soft lining materials are able to form an absorbing layer on the part of the denture in contact with oral mucosa and this allow less traumatic transmission of occlusal forces[(1)].Soft liners are often used for patients who cannot tolerate a conventional denture base [(2)]. Disinfection of denture base materials[(3)] as well as denture liners was recommended as a methods of reduction in the microbial contamination or growth and reduces oral infection as well as cross contamination[(4)]. Silver is well known for its antimicrobial activity against different positive and Gram- negative fungi and certain viruses[(5)], and recently the antimicrobial properties of nanoparticles have drawer attention of researchers[(6)]. Smaller particle size results in greater surface area to volume ratio, which enhances its chemical and biological activity[(7)]. Silver ions have been reported to inactivate important enzymes and affect the application mechanism of the DNA in bacteria .It has been reported to attach to the outer membrane and affect the permeability as well as induce structure changes in the cell –ultimately leading to cell death .In addition ,silver does not cause resistant bacterial strains to develop[(8)].The incorporation of silver–supported antimicrobial agent into denture base materials to investigate the distribution and to study

the release mode of silver ions from the base[9].In this study silver nitrate used by three volume (0.05ml,0.1ml and 0.2 ml) were incorporated into acrylic based soft denture liner to evaluate whether this addition would significantly affect some of properties of the soft lining material such as hardness, compressive strength, water sorption and solubility test.

Materials and Method

Preparation of silver nitrate solution, 0.1 N silver nitrate preparations:

_Normality (N) =weight/equivalent weight×1/ volume by litter

_Equivalent weight =molecular weight/valance

_Molecular weight of silver nitrate=169, 8731.

_Valance of silver nitrate =1.

_N=16.990/169,8731×1/1=0.10001584=0.1N

Taken (16.990±0.0001 g) silver nitrate is electronic balance. 1litter of purified water is poured into the light –proof glass bottle. The solution is prepared by pouring the pre-scaled silver nitrate into purified water; the solution prepared between 6 to 10 minutes is diluted by mixing[10,11].

Specimens grouping:-

One hundred and twenty (120) specimens were prepared from heat cure soft liner material (Vertex) to be used in this study and divided into three groups according to the test that carried out.Forty specimens for hardness test, forty specimens for compressive strength test and forty specimens for water sorption and solubility. Then each test subdivided into four subgroups according to the volume of the silver nitrate solution incorporation (0, 05 ml, 0.1ml and 0.2 ml) .Each have ten specimens.

Hardness test specimens:

A soft liner disk with the dimension of (30mm diameter, 3mm thickness)[12, 13] was used for hardness test.

Incorporation of silver nitrate with soft liner material :-

The resilient lining liquid was added to the powder and the material was mixed in accordance to the manufacturer instructions, the volume of the silver nitrate and liquid that had been added to the monomer of soft liner was taking into account and subtracted from soft liner monomer volume to achieve correct P/L ratio.

The soft liner dough was packed as a conventional method. Curing was done as instructed by manufacturer (heating up to 70 C^{O} and kept for 90 minutes then the temperature was raised up to 100c and kept for 30 minutes)[14]. A sharp blade was used to remove all the excess materials, fine grit sand paper and fine grit silicone polishing bur was used to remove the flashes .Then the specimens placed into the distilled water for seven days in the incubator at 37C°[15].

Testing procedure:-

The shore adurometer was used to measure soft liner hardness specimens .The testing value was taken as an average of different reading that were taken directly from the scale reading of durometer by using pointed dibbing tool.

Compressive strength specimens test:

A metallic molds with thickness of (12.7mm in diameter and 19mm in length was constructed for compressive strength test[16].

Testing procedure:

The specimens were subjected to compressive strength load by using compressive strength test machine. The specimens were subjected to the load with cross head speed of 0.5 mm/minute using load cell with maximum capacity 250N according to the following formula:-

Compressivestrength =F/A=(MPa)

F: Maximum load A: Cross section area

Sorption and solubilityspecimens test:-

A metallic molds of (50±1mm diameter and 0.5±0.05mm thickness) were made for sorption and solubility test[17].

Testing procedure:

After processing and finishing, all disc –shaped specimens were dried in a desiccators containing dried silica gel.The desiccators stored in an incubator at 37 c± 2c for 24 hours, after that the specimens were removed at

room temperature for one hour, and then weighted with digital electronic balance. This cycle was repeated until constant weight was determined. This was considered to be the initial weight (W_1). The specimens were immersed in distilled water for 7 days at 37 ^{0}C ±2^{0}C .After this period of time ,each disc was removed from the water with tweezers ,wiped with clean, dry hand towel until free from visible moisture ,waved in the air for 15 seconds and weighed one minute after removal from the water .This weighted represents (W_2).After that the specimens dried by the desiccators and they were weighted every 24 hours until a constant weighted (±0.5 mg) wasobtained, this weighted represents (W_3). Water sorption and solubility measured as a relative sorption and solubility in (mg/cm^2).Calculations were made according to the following formulae:

Sorption (mg/cm^2) = w_2-w_1 /surface area.

Solubility (mg/cm^2) = w_1-w_3/surface area.

Findings

Hardness test:

Table (1) showed that majority mean value are accounted in (0.2 ml) group while low level are recorded in control group,

Table (1): Descriptive statistics for hardness test

Groups	No.	Mean N/ mm	S.D.	S.E.	95% C.I. for Mean		Min.	Max.
					L.B.	U.B.		
Control	10	91.590	2.660	0.841	89.687	93.493	88.7	95.1
Silver nitrate 0.05 ml	10	95.200	0.759	0.240	94.657	95.743	93.9	96.2
Silver nitrate 0.10 ml	10	97.680	1.326	0.419	96.732	98.628	95	99.4
Silver nitrate 0.20 ml	10	101.070	2.555	0.808	99.242	102.898	97.8	106

Table(2) Games Howell (GH) test observed that most comparisons are accounted highly significant differences at "P<0.01", and significant different at" P<0.05" between 0.1 ml, and 0.2ml groups.

Table (2): (Pair wise Comparisons) by "GH" test for hardness test

Group (I)	Group (J)	Statistics		
		Mean Diff. (I-J)	Sig. (*)	critical values
Control	Silver nitrate 0.05 ml	-3.610	0.009	2.655
	Silver nitrate 0.10 ml	-6.090	0.000	2.752
	Silver nitrate 0.20 ml	-9.480	0.000	3.297
Silver nitrate 0.05 ml	Silver nitrate 0.10 ml	-2.480	0.001	1.400
	Silver nitrate 0.20 ml	-5.870	0.000	2.553
Silver nitrate 0.10 ml	Silver nitrate 0.20 ml	-3.390	0.011	2.657

Compressive Strength test

Table (3) showed that majority mean value accounted with (0.1ml) group, while low level are recorded with (0.05ml) group.

Table (3): Statistics for compressive strength test

Groups	No.	Mean	S.D.	S.E.	95% C.I. for Mean		Min.	Max.
					L.B.	U.B.		
Control	10	1.945	0.054	0.017	1.906	1.983	1.882	2.056
Silver nitrate 0.05 ml	10	1.440	0.104	0.033	1.365	1.514	1.265	1.582
Silver nitrate 0.10 ml	10	1.977	0.140	0.044	1.877	2.077	1.74	2.215
Silver nitrate 0.20 ml	10	1.459	0.085	0.027	1.399	1.520	1.344	1.582

Results of Games Howell test show highly significant differences except comparison between 0.1ml and 0.2ml groups which reported no significant different at" P>0.05" as shown in table(4).

Table (4): Pair wise Comparisons by (GH) test for compressive strength test

Group (I)	Group (J)	Statistics		
		Mean Diff. (I-J)	Sig. (*)	critical values
Control	Silver nitrate 0.05 ml	-1.105	0.000	0.352
	Silver nitrate 0.10 ml	-0.796	0.000	0.354
	Silver nitrate 0.20 ml	-0.755	0.000	0.375
Silver nitrate 0.05 ml	Silver nitrate 0.10 ml	0.308	0.000	0.085
	Silver nitrate 0.20 ml	0.349	0.002	0.200
Silver nitrate 0.10 ml	Silver nitrate 0.20 ml	0.041	0.932	0.205

Water Sorption & Solubility test

Table (5) shows that high mean value are accounted in group with (0.05 ml) while low level are recorded within controlled group.

Table (5): statistics for Water Sorption & Solubility test

Groups	No.	Mean	S.D.	S.E.	95% C.I. for Mean		Min.	Max.
					L.B.	U.B.		
Control	**10**	**3.095**	**0.356**	**0.113**	**2.840**	**3.349**	**2.4**	**3.6**
0.05 ml	**10**	**4.199**	**0.040**	**0.013**	**4.171**	**4.228**	**4.1**	**4.3**
0.10 ml	**10**	**3.891**	**0.082**	**0.026**	**3.832**	**3.950**	**3.7**	**4.0**
0.20 ml	**10**	**3.850**	**0.202**	**0.064**	**3.706**	**3.994**	**3.6**	**4.3**

Games Howell method shows highly significant differences at P<0.01, except comparison between 0.1ml and 0.2ml groups which reported no significant different at P>0.05 as shown in table (6)

Table (6): Pair wise Comparisons by (GH) test

Group (I)	Group (J)	Statistics		
		Mean Diff. (I-J)	Sig. (*)	critical values
Control	Silver nitrate 0.05 ml	-1.105	0.000	0.352
	Silver nitrate 0.10 ml	-0.796	0.000	0.354
	Silver nitrate 0.20 ml	-0.755	0.000	0.375
0.05 ml	Silver nitrate 0.10 ml	0.308	0.000	0.085
	Silver nitrate 0.20 ml	0.349	0.002	0.200
0.10 ml	Silver nitrate 0.20 ml	0.041	0.932	0.205

Discussions

Hardnesstest:-

The results of this study showed high significant increase in the mean value of hardness for experimental groupsas shown in table (1), This findings can be explained by the fact that added silver nitrate into soft liner material may effect to the plasticizer ability for softening gel formation and its ability for polymeric chains penetration, it may act fillers that increase soft liner hardness and resistance when bonded inside it, silver nitrate may act fillers that increase or decrease soft liner hardness and resistance when dispersed inside it. And high significant increase mean value of hardness for silver nitrate groups could be the result of insufficient water absorption by these groups during the elongation and higher hardness value for specimens is explained by the incorporation of silver nitrate into soft liner material which may increase stiffness and reduce the flow of the material under load [14]. This explanation is in agreement with the work of others which showed the hardness significant increased as the result of leaching plasticizer with time for soft liner material by antifungal drugs [18].

Compressive strength:-

Denture bases would have a superior cushioning effect during occlusion and mastication .Many of these forces are compressive in nature ,and therefore effect of compression load on the test materials must be evaluated. In the methodology of this research ,determination of the compressive strength of the experimental material in compression with the control material was established ,the material were prepared in a cylindrical discs . During testing compressive load continued to compress

cylindrical discs, this design of testing is simulating in a degree compressive load during function of occlusion or mastication [12]. Statistical analysis showed high significant differences regarding ultimate compressive strength and deflection value .Higher compressive strength value recorded by the experimental material may give an indication of the rigidity in comparison with control material. Silver nitrate that is recorded by the experimental material indicated more rigid properties. By this study, the ultimate compressive strength as will showed that the compressive strength of the experimental material is significant high in (0.1ml) concentration than that of the control group material (table 3).The lower deflection value recorded by the experimental material indicated more rigid properties, this is attributed to the presence of silver nitrate polymer which made the experimental material more rigid. The compressive strength of the experimental material is high significant increase after 0.1ml concentration showed that of control material, but 0.05-0.2ml is high significant decrease with the control group (table 4). Lower compressive strength value recorded by the experimental material give an indication that the silver nitrate reduced water sorption and gave more rigidity to the experimental material in comparison with control material. This study is agreement with others work that showed reduce the molecular binding force between the reactant molecules and allows greater deformation upon stretching or flexion through exhibiting multiple micro fracture that weaken the silver nitrate loaded resin samples[19].

Water sorption and solubility:-

The results of sorption test showed that an increase in the mean value of soft liner material after incorporation with silver nitrate (table 5). This result obviously is due to leaching out of water –soluble ingredients, residualmonomer ,plasticizer and initiators from the soft liner in addition to silver nitrate ,during immersion in distilled water ;as a result there will be more micro porosity which will be eventually filled by water [20]. The findings of this study was done by incorporation of silver nitrate into soft liner material result high significant increase in mean value compare with control group(table 6).The chemical interact between soft liner material and silver nitrate explain the high water sorption of soft liner material after addition silver nitrate this is in agreement with the other work who suggested that variation in chemical composition could create some of structural spaces that might lead to higher water up take[21] .

Conclusions

Silver nitrate incorporation was significantly increases the hardness of soft lining material..

Compressive strength was significantlyincreased after incorporation of 0.1 ml of silver nitrate while incorporation of 0.05 and 0.2 ml of silver nitrate significantly was decreased.

Water sorption and solubility were significantly increased after incorporationof silver nitrate in all experimental groups.

Conflict of Interest: No

Source of Funding: Self

Ethical Clearance: No

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Evaluation of Transaminases,Total Bilirubin and Ferritin in Iraqi Thalassemia Patients

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Abstract

Background: Thalassemia is a hereditary disease which characterize by a severe form of anemia occurring early in life associated with splenomegaly and bones changes.

Aim: The aim of this study was to evaluate the serum levels of ALT (Alanine transaminase),AST (Aspartate transaminase),bilirubin and ferritin in Iraqi β-thalassemia patients.

Subjects and Method: Fifty Iraqi patients diagnosed clinically by hematology doctors as β-thalassemia major(TM) were enteredin this study, they conducted to Ibn AL-Baladi hospital/Baghdad during the period from December, 2017 to march, 2018.The age ranges of patients were (6-36) years,twenty healthy age match were used as control group(Cot). Blood samples (3-5) ml, were collected from the two groups under study TM and control group.Serum was separated, drawn in two plane test tubes, and kept frozen (-20 ◦c) till the use. Ferritin levels were measured by using Ferritin (Kit Ref 04491785 of cobas e 411 analyzer) according to the manufacturing company. Total Serum Bilirubin concentration(TSB),Transaminases (GOT&GPT) activity were also measured by colorimetric methods, according to ELITech kit,Ref. BITO-0250 France for Bilirubin,and according to ELITech kit,Ref.ALSL-0455 France, for Transaminases/ France,using flexor 150 apparatus.

Findings: Results indicated that serum GOT and GPT were significantly elevated in TM patients (47.560 ± 30.472) U/L and (58.755± 5.369) U/L respectively in comparisons to their levels in the control group (19.940 ±9.427) U/L and (8.231 ± 3.830) U/L respectively,also a significant increase in serum Ferritin concentration (2668.±1775.436) ng/dl in TM patients in comparison with control group (31.000±4.735) ng/dl,with an elevated levels in mean value of serum TSB in thalassemia patients (34.76 2 ± 18.526) in comparison to control group (8.231 ± 3.830) .

Conclusion: Increase ALT (Alanine transaminase), AST (Aspartate transaminase), bilirubin and ferritin inthalassemiapatients may refer to liver dysfunction.

Keywords: *Beta thalassemia major (TM), ALT (Alanine transaminase), AST (Aspartate transaminase), bilirubin and ferritin.*

Introduction

Thalassemia is a form of inherited autosomal recessive blood disorder which it characterized by abnormal formation of hemoglobin[1]results in the adequate oxygen transport and the destruction of red blood cells inthe bone marrow (ineffective erythropoiesis) and the peripheral circulation (hemolysis)[2]In normal cases,most of adult hemoglobin (HbA) is consist of 4 protein chains, two α and two β globin chains which arranged in a hetero- tetramer. Thalassemia patients have defects in either the α or β globin chain, causing production of an abnormal red blood cells[3]due tothe variant or the missing genes that affect hemoglobin(the protein in red blood cells that it carries oxygen) synthesis. The people with thalassemia have less hemoglobin and also have fewer circulating red blood cells than normal which result in mild or sever microcytic anemia[4]. Thalassemia word was derived from the Greek word *thalas*which means sea and *emia*that stands for the blood

anemia, indicating its prevalence in the regions around the Mediterranean Sea[5].For many patients who they were with chronic anemia,regular blood transfusions have affectively extended life suspense in thalassemia major patients [6]Blood transfusions contain red cells that reach the end of their life due to the aging or defects they are broken down, and then hemoglobin molecule broken up and the iron recycled. Humans are unable to eliminate the excess iron and regulate the body iron stores by limiting absorption [7]

Too much iron in the body of human is called iron overload [5]Iron was transported as transferrin to the liver or the spleen where it was stored as ferritin[8]. Serum ferritin levels were measured in patients as a part of the iron studies workup for the anemia. Ferritin is also used as a marker for the iron overload disorders, such as hemosiderosis, hemochromatosis and porphyria in which ferritin level may be abnormally raised[9] Iron accumulation (ferritin) in the liver and the heart worsening of hepatic fibrosis, and development of the cardiac fibrosis [10]

Beta-thalassemia major patients suffer from many problems rather than severe anemia including; increased susceptibility to bacterial infections which plays the major role in the patient's morbidity and mortality[11] Degradation of the hemeafter 120 days in the circulation to produce the green pigment biliverdinand the bilirubin. Bilirubin binding to the albumin and enters a hepatocyte then excreted to the bile.Bilirubin can be measured as direct or conjugated. Sometimes, total amount of the bilirubin in the blood measured [12]

Alanine Transaminase (ALT) which called also serum glutamate -pyruvate transaminase (SGPT). Alanine Transaminase is present in high concentration in the liver lesser extent in skeletal muscle, heart and kidney[13], measurement the activity of ALT in serum is used as an indicator of hepatocellular damage [13] Aspartate Transaminase (AST) enzyme is called also glutamate oxaloacetate transaminase (SGOT), which present in high concentration in the following organs: heart, skeletal muscle, liver, kidney and erythrocytes[14] The damage to any of these tissues may be result in the increased plasma AST [15].

The study was aimed to determination of serum transaminase (ASOT, GPT), ferritinand the total serum bilirubin levels in β-thalassemia patients receiving multiple blood transfusions.

Materials and Method

Subjects

Fifty Iraqi patients of both sexes (28 male and 22 female)which were diagnosed clinically with β-thalassemia major were participated in the present study, they conducted to Ibn AL-Baladi hospital during the period extended from December,2017 to March, 2018. Their age ranges were (6-36) years old; all the patients were having chelating therapy (desferrioxamine). Twenty healthy age match were used as a control group.

Samples collection

Blood samples (3-5) ml, were collected from the patient group and healthy control group, left to stand for an hour then centrifuged(2500r pm) for fifteen minutes . Serum was separated, drawn in two plane test tubes, and then kept frozen (-20 ◦c) till the testing day.

Determination of serum ferritin levels

Ferritin levels were measured by using Ferritin Kit Ref 04491785 of cobas e 411 analyzer according to manufacturing company (Roche Diagnostic).

Determination of serum liver function

Total Serum Bilirubin concentration (TSB),Transaminases (GOT&GPT)activity and Creatinine were measured by the colorimetric methods,according to the ELITech kit,Ref. BITO-0250 for Bilirubin,and according to the ELITech kit,Ref. ALSL-0455, for Transaminases, and according to the ELITechkit. Ref, CRCL-0250 France for Creatininedetermination,by using flexor 150 apparatus.

Statistical Analysis

Statistical analyses for this study done by using Microsoft office (SPSS version 10.01). The data were present as mean ±SD. Alsothe t-test for two independent means, *P* value 0.05 levels or less considered as a significant result.

Finding and discussion

In this study Fifty Iraqi patients were diagnosed clinically with the β-thalassemia major wasparticipated, they conducted to the Ibn AL-Baladi hospital during the period extended from December, 2017 to march, 2018.

Their age ranges were (6-36) years old; twenty healthy age match persons were used as a control group.

About (3-5)ml of the blood were collected from the two groups,and serum was used for the detections of transaminase [Glutamic Oxaloacetic (GOT),Glutamic Pyruvic Transaminase (GPT)]Total Serum bilirubin and the ferritin levels in both groups.

Table (1) showed findings of Ferritin,total serum bilirubin, and transaminase expressed as mean ±SD in 50 patients with the major thalassemia(TM) and 20 control group(Cot). As seen in Table(1)and figure (1) there was a significant increase in the serum Ferritinconcentration (2668.±1775.436) ng/dl in TM patients in comparison with the control group(31.000±4.735) ng/dl,(P≤0.05).

TSB titers were elevated in the TM patients(34.76 2 ± 18.526) mm/l compared with the control group(8.231 ± 3.830)mm/l,(P <0.001) table(1)and Figure (2).

Serum GOT and GPT were significantly elevated in the TM patients(47.560 ± 30.472)U/L and (58.755± 5.369)U/L, respectively in comparisons to their levels in the control group(19.940 ±9.427)U/Land (8.231 ± 3.830)U/L respectively,Table (1), Figure (3) and Figure (4).

Table (1):Mean±SD values of serum ferritin,TSB,GPT and GOT in β the thalassemia patients group (TM), and the control group (Cot).

Parameters	Cot(I) Mean±SD	TM(II) Mean±SD	p-value
Ferritin ng/dL	31.000±4.735	2668.880±1775.436	<0.001
TSB mm/L	8.231 ± 3.830	34.76 2 ± 18.526	<0.001
GPT U/L	17.600 ± 4.654	58.755± 5.369	0.0147
GOT U/L	19.940 ±9.427	47.560 ± 30.472	0.0065

TSB=Total serum bilirubin, Glutamic Oxaloacetic (GOT),Glutamic Pyruvic Transaminase (GPT).

From FigureS (1),(2), (3) and (4) we showed that the highly significant differences in TSB,GOT,GPT and serum Ferritin between the β-thalassemia infected group and the control group.

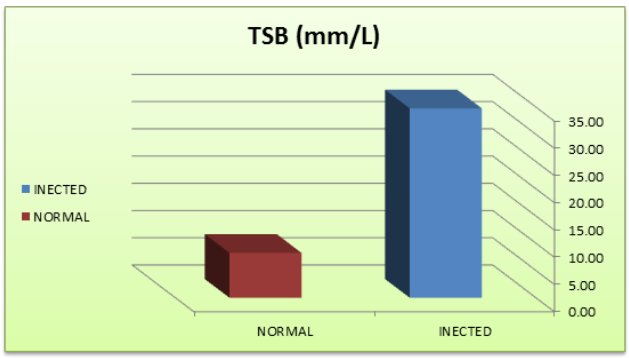

Figure -1: Mean differences of Total serum bilirubin(mm/L) between the thalassemia infected patients and the control .

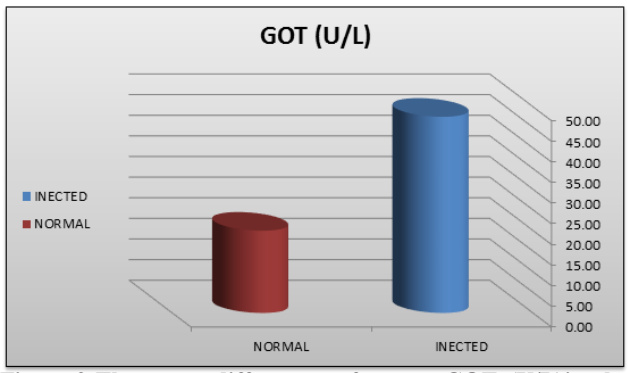

Figure-2:The mean differences of serum GOT (U/L)in the thalassemia infected patients and the control .

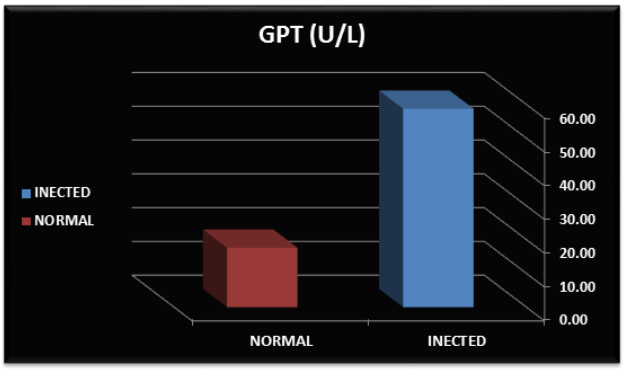

Figure -3:The differences of GPT value (U/L) in the normal and the thalassemia patients expressed as Mean±SD.

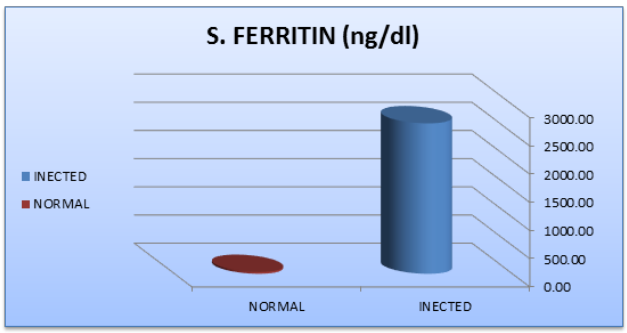

Figure -4: Mean value of serum ferritin level(ng/dl) inthe β-thalassemia patients and the control.

The findings showed significant increase ($p<0.05$) inthe ferritin levels ofthalassemia patients comparing with the healthy control group. Iron initially stored as ferritin, is deposited in organs as hemosiderinwhich is toxic to the tissue.Most humans avoid iron overload solely by regulating the iron absorption. Those who cannot regulate the absorption well enough get complaints of iron overload. In these diseases, the toxicity of the iron destroyed the body's ability to bind and store iron. Patients with thalassemia major accumulate body iron over time as a consequence of the continuous red blood cell transfusions which cause endocrine, hepatic and cardiac complications [16,17,18]. all of these studies are associated with the current study results.

The total bilirubin level measured sum of direct bilirubin and indirect bilirubin, when the total bilirubin level increased this indicted that increased both or only one of them [19]. Bilirubin, is the first point that helps tothe recognized jaundice,hemolysis induces deposition of the bilirubin causing intrahepatic cholestasis and the cholelithiasis[20,21]. This study had demonstrated a higher mean value of TSB in the thalassemia patients (34.76 2 ± 18.526) in comparison to its value in the control group(8.231 ± 3.830).These findings were disagreed with another study done by Chakravarti*et.al*, 2005 [22]. which found that the bilirubin levels were not significantly altered in these patients. The study findings were similar to the other findings reported by Sarkis, (2000)[23]. who stated that there was a higher mean value of the total serum bilirubin and a highly significant difference between the patients with HCV positive andthe patients with HCV seronegative.

The study results had demonstrated that the mean value of GPT, was (47.560± 30.472)U/L in the thalassemia patients, compared to (19.940 ±9.427)U/L in the control cases,and the difference statistically was significant ($P<0.002$). This was compatible with the other studies Wanachiwanawin*et.al*, 2000 [24]. which reported that the thalassemia patients had significantly abnormal level of the serum GPT.

Shindo*et.al,*1995 [25]. found that patients of anti-HCV positive who have elevated serum GPT level are more likely to have a significant liver disease than those who have normal level of serum GPT. Serum GPT elevation in anti-HCV positive individuals suggests the presence of the liver damage such as viral replication [25]. and iron overload .The study results have also demonstrated that there was significant difference in the GOT between the patients and the control group this findings was similar to Al Hawsawi,2000 [26] found that there was a highly significant difference in the GOT level between thalassemic patients with HCV seropositive and patients with HCV seronegative.

The thalassemic patients have a greater risk of liver damage (as indicated by the elevated aminotransferase and the serum bilirubin) compared to the control groups [27]. probably resulting from the infection, cholestasis, hemosiderosis or as a result of congestion due to the heart failure.

In addition, seropositive thalassemic patients have significantly higher levels of the aminotransferase enzymes and the serum bilirubin. This can be explained either by direct damage of the hepatocyte by viral invasion or by the development of an immune complex. Consequently it is necessary in these patients to measure the aminotransferase levels monthly for six months; if there is persistent elevation, it should be to considered as chronic hepatitis and liver biopsy is indicated[28].

Conclusion

Our results showed significant increase in the ferritin levels with higher mean value of TSB and GPT of thalassemia patients comparing with the healthy

control group.

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Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: All experiments were carried out in accordance with approved guidelines.Experimental protocols were approved under the Biology Department, College of Science, Mustansiriyah University, Iraq and

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Evaluation the Effect of Black Seed Oil and Taramira Oil on the Hardness and Surface Roughness of Heat Cure Acrylic Resin Denture base Material

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Abstract

Background: Separating medium is a layer of coat which is applied to a mouldsurface to avoidthe adhering the second surface to the first surface. The mould must be coated during acrylic resin processing to inhibit liquid resin from penetrating into the gypsum and water from gypsum leaking to acrylic resin. For several years, tin foil was the most acceptable separating medium, and because it's challenging to apply a tin-foil substitute, a cold mold seal is utilized.

Objective of this study: The aim of thecurrent study was to assess the effect of black seed oil and taramira oil on surface roughness and hardness of heat acrylic resins.

Materials and Method: Sixty samples were made from the heat acrylic resins and divided into two categories (30 samples in each categories) depended on test; surface roughness and hardness, each categories contain three groups: group 1;(10) samples of heat acrylic resins treated with cold mol seal (control group), group2; (10) samples of heat acrylic resins treated with black seed oil and group3;(10) samples of heat acrylic resins treated with taramira oil. The average of the hardness of the specimens has been determined with using the Shore D hardness device¸ also the average of the surface roughness of the specimens has been determined with using profilometer device.

Findings: The results showed that there was a statistically significant difference in the hardness and non-significant difference in surface roughness of heat acrylic resinsamong the testing groups.

Conclusion: In this study concluded that black seed oil gives more hardness in comparison with cold mold seal and taramira oil; also black seed oil gives more smoothness (less surface roughness) followed by taramiraoil.It is concluded, that black seed oil and taramira oil could beutilized as a separating medium.

Key wards*: Denture base,acrylic resin,black seed oil,taramira oil,cold mold seal.*

Introduction

Polymethylmethacrylate (PMMA) is the most popular dental material used in the construction of removable prostheses, it is necessary to apply separating medium to the gypsum surface before packing of PMMA[1].

Separating medium is a "coat of layer applied to surface to prevent a second surface from adherence to the first surface"[2]. A layer of gypsum impregnated with polymer remains would be attached to the denture surface If the mold surface is not separated with a separating material leading to an unaesthetic and poorly fitting denture base [3].Thus; the separating medium must be applied to the surface of the mold [4].

Many materials are used for dental stone separation; cold mold seal is one of commonly used separating medium. The literature indicated thatthe tin foil separating medium is the best, but it istime-consuming and difficult to apply. Consequently, the use of other separating medium has been developed[5].

Wally in 2014[6] used olive oil and found that non-significant difference between cold mold seal and olive oil regarding tensile strength of heat acrylic resins. While Mohammed in 2014[7] studied the influence of glycerin on the hardness of heat cure resin and found that glycerin gives highly hardness than cold mold seal. Altaie et al., in 2015[3]used olive oil, tin foil and cold mold seal as separating medium and found that using olive oil not affect on hardness of heat cure acrylic resin.

The purpose of this study was to evaluate the effect of black seed oil and taramira oil as a separating medium on hardness, and surface roughness of heat cure acrylic resin denture base materials

Material and Method

Samples grouping:

60 samples were made from heat cure acrylic resin (Superacryl Plus, Colombia)**;** Specimens were grouped into: 30 specimens made for hardness testand these where subdivided into:

*(10) samples of heat cure acrylic treated with cold mold seal (control group);

(Switzerland).

*(10) samples of heat cure acrylic treated with black seed oil, (Kingdom Saudi Arabia),

*(10) samples of heat cure acrylic treated with taramira oil **(**Pakistan).

30 samplesmade for surface roughness test and these where subdivided into:

*(10) samples of heat cure acrylic treated with cold mold seal (control group).

* (10) samples of heat cure acrylic treated with black seed oil.

*(10) samples of heat cure acrylic treated with taramira oil**.**

Preparation of the specimens:

Two metal patterns (stainless steel)were customized into desired shape and dimension using aturning machine:

A- Surface hardness test with dimension of (65 mmx10 mm x2.5mm) length, width and thickness respectively [7]**,** figure (1-A).

B- Surface roughness test with dimension of (80mm x 10mm x 2.5mm) length, width and thickness respectively [8],figure (1-B)

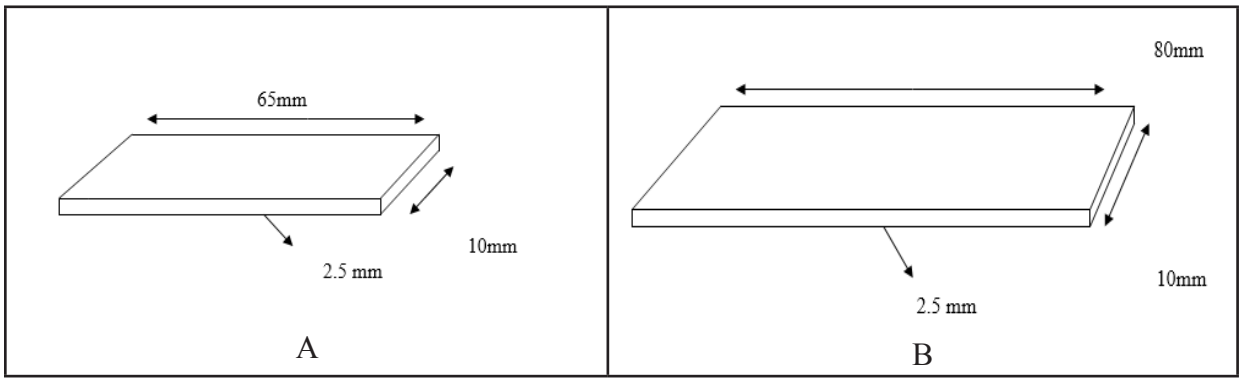

Figure (1):A-surface hardness test B- surface roughness test

The 2 metal pattern werelayered with separating medium.The lower part of a flask was covered with dental stone(Durguix, Spain). Once complete set of the stone surface, bothmetal patterns and stone were separated withcold mold seal. The upper part of the flask was positioned over the lower part, andthen filled with stone. The flask was left for 60minutes, after that the upper and lower parts were opened carefully and patterns were removed.A disposable syringewas utilized to measure 2cc of separating media (cold mold seal, black seed oil and taramira oil). The measured separating medium was applied onto the stone surface by a fine brush [9]. This

technique of applying material (cold mold seal, black seed oil and taramira oil) was repeated with all moulds of the specimens in this study.

Heat cured acrylic resin was mixedandpacked according to manufacturers instruction,and then the flask was positioned under the hydraulic pressand the pressure was applied slowly in order to make sure even flow of the acrylic dough throughout the mould space. Thenthe pressure was released and the flask was opened and the excess material (flash) was taken away. Then the two halves of the flask were finally put in contact and left under the press (1500psi) for 5 minutes. Then the flask was placed in a flask clamp.According to ADA specification, No.12 (1999), short curing cycle was conducted[10]. the flask was left to cool for thirty minutes at room temperature and then with tap water for 15 minutes. All acrylic specimens were then taken from the stone mould.

Acrylic patterns of surface roughness were cut into equal rectangular plates with an acrylic separating disk to obtain the final measurement of 20mmlength x10 mmwidthx2.5 mm thickness. Thethickness of 2.5mm represents the average thickness of acrylic denture base, while the length and width coincides for suitable measurements in the surface roughness tester [8].To remove all flashes of acrylic,the acrylic bur was utilizedfollowed by using sand paper (120 grain size)[9] in order to get smooth surface for the specimens used for hardness test. On the contrast, the specimens for surface roughness test, are not be polished.All specimens were kept in distilled water at 37Cº before testing [10], figure (3).

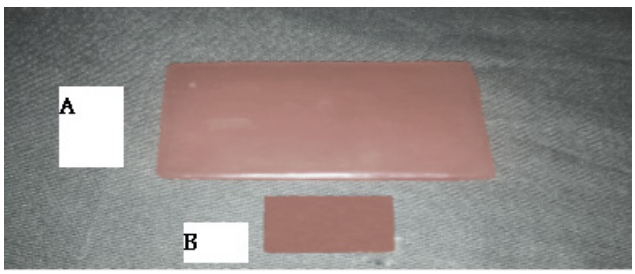

Figure (2) :(A): Hardness specimen. (B): Surface roughness specimen

Shore D hardness tester device (Italy) was used in this study for measuring procedure, figure5.According to the device instruction the hardness value is determined by the penetration of the Durometer indenter into the specimen. Three reading was done on different areas of each specimen and the mean of the three reading was calculated.

Figure (3): Shore D hardness tester device.

The surface roughness of each specimen was measured by using profilometer surface roughness device, figure (4). The profilometer was supplied with stylus made of diamond; the specimen's surfacewas fixed in a flat position to the base of the profilometer by glue. The stylus was moved for a distance of 1.7 mm across thesurface of the specimen in three different directions depending on apparatus design. Three reading was recordingfor each specimen, and the mean value for each specimen was the average of the reading. Then the data was collected and subjected to statistical analysis.

Figure (4): Profilometer surface roughness device

Findings

Surface hardness test**:**

All values of mean and standard deviation for each group is listed in table (1). The highest mean value is (79.340) for black seed oilgroup, followed by the taramira oil group which is (77.400), while the control group showed the lowest mean value (74.720), figure (5).

Table (1): Descriptive statistics test for surface hardness of studied group

Studied group	N	Mean	SD	SE	Min.	Max.
Control	10	74.720	1.6 80	.495	71.54	79.44
Black seed oil	10	79.340	2.196	.594	74.80	80.67
Taramira oil	10	77.400	1.540	.400	73.90	81.85

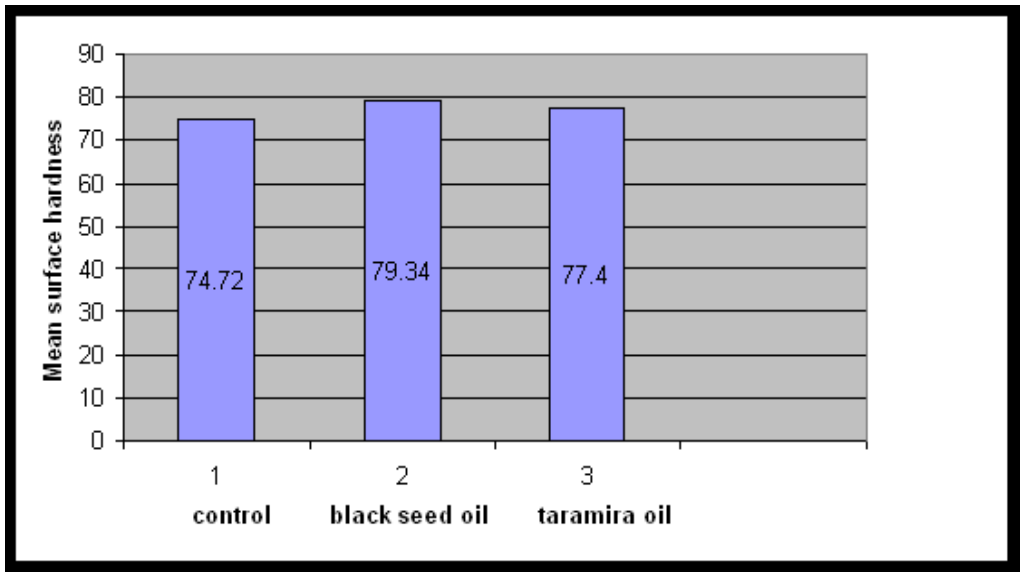

Figure (5): Bar chart of mean of surface hardness test

The ANOVA test indicated that there were significant differences for surface hardness among all groups as demonstrated in Table (2) where (P<0.001).

Table (2): ANOVA test for surface hardness among studied groups

	Sum ofSquares	**df**	**Mean Square**	**F**	**Sig.**
Between groups	107.635	2	53.817	10.866	.000 (HS)
Within groups	133.710	27	4.953		
Total	241.345	29			

Furthermore**,** therewas a highly significant difference (P<0.001) between each groups as demonstrated in table 3(The LSD test).

Table (3): LSD test for studied groups

Studied Groups P-value		**LSD (test)**	
		Sig.	
Control	Black seed oil	0.000	HS (P<0.001)
	Taramira oil	0.000	HS (P<0.001)
Black seed oil	Taramira oil	0.000	HS (P<0.001)

Surface roughness test:

The values of mean, standard deviation, standard error, minimum and maximum of surface roughness for all groups are list in table (4). The control group created the highest value of mean (1.349μm), while the black seed oil group produced the lowest value of mean (1.346μm), figure (8).

Table (4): Descriptive statistics test for surface roughness of studied group

Studied group	N	Mean(µm)	SD	SE	Min.	Max.
Control	10	1.349	0.241	0.073	1.020	1.610
Black seed oil	10	1.346	0.223	0.071	1.011	1.659
Taramira oil	10	1.347	0.232	0.096	1.077	1.660

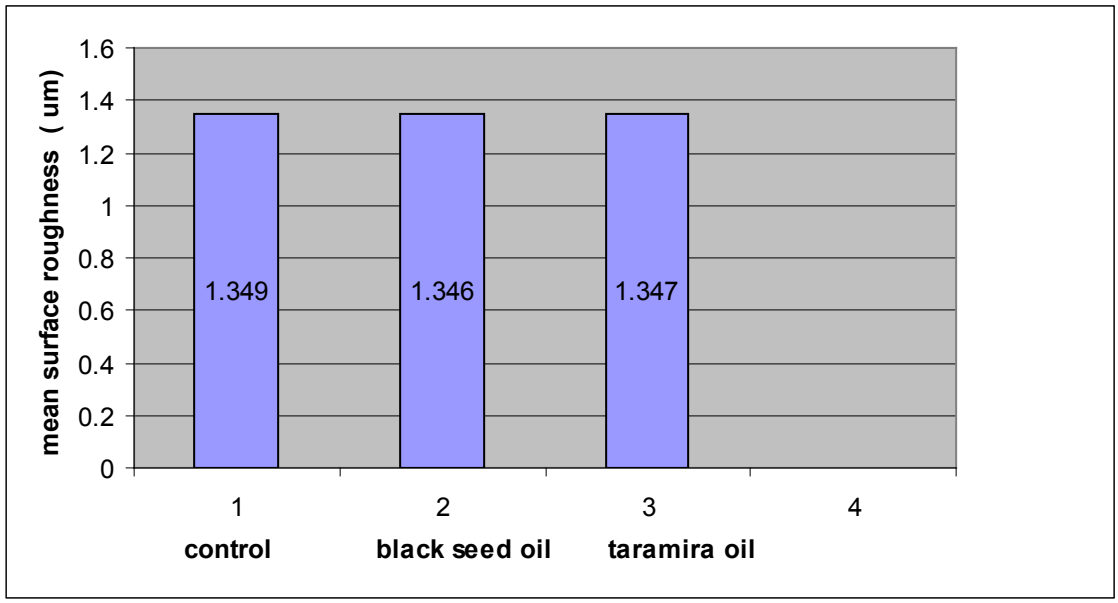

Figure (6): Bar chart of mean of surface roughness test

Among all groups , there were no significant differences as demonstrated in Table (5).

Table (5): ANOVA test for surface roughness

	Sum of Squares	df	Mean Square	F	Sig.
Between groups	.000	2	.000	.000	1.000 (NS)
Within groups	1.799	27	.067		
Total	1.799	29			

Discussion

Separating medium is considered as the mainfeature which influence thesuccess of dental because of its effect on the mechanical and physical properties of acrylic resin. For dental stone, many substances areutilized such as cold mold seal [11].

In this study, black seed oil and taramira oil used as separating materials with cold mold seal (control group) to evaluate their effect on the hardness and surface roughness of acrylic denture base material.

Surface hardness test:

The results of the current study revealed thatthe black seed oilgroup created the highest mean value followed by the taramira oil group, while the control group showed the lowest mean value (table 1), also the result showed highly significant difference among them (table 2,3). Black seed oil andtaramira oil improved the hardness of the acrylic resins, this could be explained

by water sorption phenomenon of methyl methacrylate denture base material that is mean the cold mold seal film is not fully water eliminator and this inhibit complete polymerization of acrylic [12,13]. Black seed oil showed highly significant difference than taramira oil this could be due to chemical composition of the major unsaturated fatty acids were linoleic acid followed by oleic and lignoceric acid in chemical composition of the black seed oil [14]. This result agrees with Hatim et al., [15] who found that addition of black seed and thyme oils improved the transverse strength and hardness of acrylic resins. Altaieet al., [3] used olive oil as separating medium, and revealed that therewere no significant differences between olive oil andcold mold seal on hardness of acrylic resin.

Surface roughness test:

Smooth surface of acrylic resin resists the buildup of stain, debris and plaque. Increase surface roughness has a detrimental effect on the aesthetic of the denture and health of oral tissues [4].

The results of the current study indicated that the cold mold seal (control) group hadgreatest mean value of surface roughness followed bytaramiraoil group, while the black seed oil group showed the lowest mean value (table 4), this decrease in the mean value of surface roughness may be due to the nature of oil which makes the surface smooth [3]. Also the result showed that there is a non significant difference among groups (table 5). This could be related to the present of glycerin in the composition of cold mold seal that is similar to the chemical oil nature of black seed and taramira oils [4].

However, there is no previous studies in this field to correlate the results of this study (hardness and surface roughness test) with it.

Conclusion

From the present study the following conclusion can be with drawn:

1-Black seed oil gives more hardness in comparison with cold mold seal, andtaramira oil which is gives more hardness than cold mold seal.

2- Black seed oil gives more smoothness (less surface roughness) in comparison with taramira oil and cold mold seal.

3- Instead of cold mold seal, black seed and taramira oils can be utilized as the separating medium

Conflict of Interest: None

Source of Funding: self

Ethical Clearance: None

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G- Protein Coupled Receptor Purification from Whole Blood of Iraqi Diabetic and Diabetic Nephropathy Patients

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Abstract

The study aimed to purified G- Protein Coupled Receptor (GPCR) from the whole blood of control ,G1, diabetic,G2, and diabetic nephropathy ,G3,patients by different chromatography techniques for the first time in world that may be important in early detection and active treatment in these patients.

The GPCR extracted from whole blood by precipitation of proteins with ammonium sulfate,then purified by ion exchange chromatography, as a first step using DEAE-Sepharose .A gel filtration chromatography was applied as a second step of purification using Sepharose 6B.The molecular weight and purity of GPCR was determined by electrophoresis.

Results of ion exchange chromatography for G1 showed two proteins peaks that appeared after elution by the gradient concentration of sodium chloride. Data indicated that GPCR is located in the first protein peak at fraction numbers between (6 and12). Results for G2 showed two proteins peaks that appeared after elution with the gradient concentration of sodium chloride .Then, fractions were collected and concentrated to be applied in gel filtration chromatography.Results showed a single active protein peak after eluted with elution buffer that was identical with the peak that presented GPCR concentrations at fractions number (14)for control group and show of single active protein peak that was identical with the peak that presented GPCR concentrations at fractions number (12)for G2and G3 .

The molecular weight of GPCR that estimated byelectrophoresis that two bands of GPCR appear in G1at (~ 30 KD) and (~ 51 KD) .Results ,also , showed appearance of three bands in G2 related to GPCR in (~ 29 KD) and (~ 47,49 KD) .

The conclusion could be drawn from this studythat GPCR appear in groups is a marker in detection and treatment of diabetic and diabetic nephropathy.This finding could be useful in the early detection and active treatment of diabetic and diabetic nephropathy patients based on type of GPCR that be identified .

Keywords: *GPCR Purification, Whole blood , Diabetic , Diabetic Nephropathy.*

Introduction

The (GPCRs) are receptors encoded by the human genome.Several GPCRs have been identified as a potential therapeutic targets for the treatment of diabetes and diabetes complications, including retinopathy, nephropathy, and neuropathy [(1,2)].

Study ,revealed that GPCRs are expressed in the pancreatic islets and may play an important role in the normal glucose homeostasis [(3)].

Many GPCRs are directly involved in the development of insulin resistance and β-cell dysfunction that can lead to T2DM[(4,5)] . Sweet taste receptor appear to dominate postprandial glucose-dependent insulin and GLP-1 release [(6)] .

Glucose-dependent stimulation of insulin secretion is observed with$G_{\alpha s}$and $G_{\alpha q}$-coupled receptors linked to protein kinase C activation and inositol triphosphate which linked to insulin secretion [(7,8)] .Researches demonstrated that these receptors are expressed in high

levels in pancreatic β-cells [9] .

Purification is a major challenge in the field of GPCR research because many GPCRs are denatured by detergents (due to the difficulty of purifying GPCRs) [10,11] .So,this study designated to purified GPCR from the whole blood of control, diabetic and diabetic nephropathy patients.

Material and Method

1- Extraction of GPCR from wholeblood :

One hundred fifty individuals with age ranged between (40-65) years were enrolled in this study.They divided into three groups (N=50 per each group) as follows: group 1(G1) represent control group .Group2 (G2) represent diabetic patients andgroup 3 (G3) represent diabetic nephropathy patients.

Blood that obtained from subjects were centrifuged at 2500g for 15 minutes to separate pack cell volumes . Supernatant plasma and buffy coat were discarded . RBCs were washed with (3) volume of normal saline and hemolysis by adding (5) volumes of water . After 20 minutes , hemolysis was centrifuged at 3500g for 20 minutes . Supernatant was collected and filtered through qualitative filter paper (whatmen-1) to remove left out cell debris . It was followed by precipitation of proteins with 50 – 75% ammonium sulfate . For precipitation an equal volume of saturated solution of $(NH_4)_2SO_4$ was mixed with filtrate and after 15 minutes , it was cooled and centrifuged at 3500g for 10 minutes . Finally , the pellet was dissolved in a minimum volume of (1.5 mM) of Phosphate Buffer Saline pH 7.3 .The Pellet cells were thawed and suspended in extraction buffer (1.5 mM PBS buffer, pH =7.3, 1 mM AEBSF) and then protease inhibitor cocktail (up 10 ml for 1ml) was added with gentle swirling on ice. The DDM(n-Dodecyl-B-D-Maltoside) (1%) was added to the lysed sample and stirred on ice for 1 hour.The dissolved pellet was dialyzed for 72 hours against 1500 ml of dialysis buffer (1.5 mM PB, pH 7.3) in a cold room .buffer was changed three times during 72 hours.

Protein concentration was estimated according to the Bradford method (1976)[12].

GPCR concentration was estimation by ELISA [13] using kit from Blue Gene Biotechnology, China .

2 - G-protein Coupled Receptor Purification:

GPCR that extracted from blood was purified by using ion exchange chromatography as a first step of purification, and gel filtration chromatography as a second step of purification.

2.1- Ion Exchange Chromatography[14]:

Five ml of crude extract was applied on the DEAE-sepharose column (2.5 x 8) cm . The column was equilibrate and washed with an equal volume of 50 mM of phosphate buffer saline contained DDM (pH=7.4) to wash uncharged and positive charged proteins in protein mixture of crude GPCR . The bound proteins were then eluted using NaCl (0.1- 0.5) M . Fraction were collected in 5 ml tube at a flow rate of 0.5 ml/min and eluted with gradient (0.1 – 0.5) of sodium chloride solution .Then GPCR crude was eluted with buffer.The fractions that gave the highest absorbance were collected. Protein and GPCR concentrations were estimated for each fraction .

2.2-Gel filtration chromatography[14] **:**

Aliquot of five mL of concentrated fraction was injected into sepharose 6B column (65 x 1.5) cm which previously equilibrated with 1.5 mM phosphate buffer saline (pH=7.4) , and eluted with elution buffer PBS pH =7.4 containing (0.5Mm) DDM . Flow rate was (1ml / min) with 5 ml for each fraction that monitored at 280 nm .Protein and GPCR concentration were determined for each fraction.Fractions which gave the highest absorbance and concentration of GPCR were collected.

3- Estimation of GPCR Molecular Weight [15]**:**by SDS-PAGE Electrophoresis .

Findings

Table (1) shows protein and GPCR concentrations for extraction and solubilization steps of lysate . Results show a decrease in protein and GPCR concentrations after extraction and solubilizationsteps .

Table (1): protein and GPCR concentrations for extraction, solubilization and precipitation steps of lysate

Control

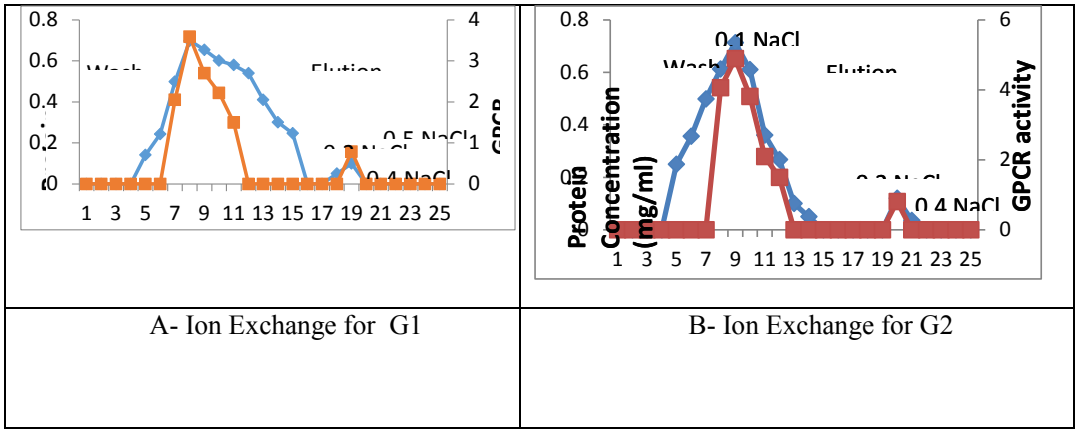
Sample	**Volume(ml)**	**Protein con.(mg/ml)**	**GPCR con.(ng/ml)**
Crude	30	0.89	6.61
Precipitate by $(NH_4)_2SO_4$ (50-75%)	15	0.718	5.34
Diabetic			
Sample	Volume(ml)	Protein con.(mg/ml)	GPCR con.(ng/ml)
Crude	30	0.92	6.19
Precipitate by $(NH_4)_2SO_4$ (50-75%)	15	0.799	6.052
Diabetic nephropathy			
Sample	Volume(ml)	Protein con.(mg/ml)	GPCR con.(ng/ml)
Crude	30	0.94	5.322
precipitate by $(NH_4)_2SO_4$ (50-75%)	14	0.801	4.95

Non –ionic detergent ,dodecal-β-D-maltoside (DDM) was used in the extraction and solubilization of GPCR based on previous study[16,17].

Purification of GPCR Extracted fromf blood

Ion Exchange chromatography[18,19]:

Results of G1 showed two proteins peaks ,figure (1-A) that appeared after elution by the gradient concentration of NaCl , while no protein peaks appeared in the washing fractions . The GPCR concentration were measured in the fractions of these two protein peaks . Data indicated that GPCR located in the first protein peak (eluted at 0.1 M of NaCl) at fraction numbers between (6 and12) . The second peaks (eluted at 0.2 M of NaCl) gave a peak between (18-20), thus its neglected .



A- Ion Exchange for G1 B- Ion Exchange for G2

Figure(1) : Ion Exchange for A- G1 B-G2

Results for G2 , figure (1-B), showed two proteins peaks that appeared after elution with the gradient concentration of sodium chloride , while no protein peaks appeared in the washing fractions . Data indicated that GPCR located in the first protein peak (eluted at 0.1 M of NaCl) at fraction numbers between (7 and13) . The second peaks (eluted at 0.2 M of NaCl) give peak between (18-20) .

Results for G3 in figure (2) showed two proteins peaks that appeared after elution by the gradient concentration of NaCl , while no protein peaks appeared in the washing fractions . Data indicated that GPCR located in the first protein peak (eluted at 0.1 M of NaCl) in fraction numbers between (7 and13) . The second peaks (eluted at 0.2 M of NaCl) give peak between (19-21) .

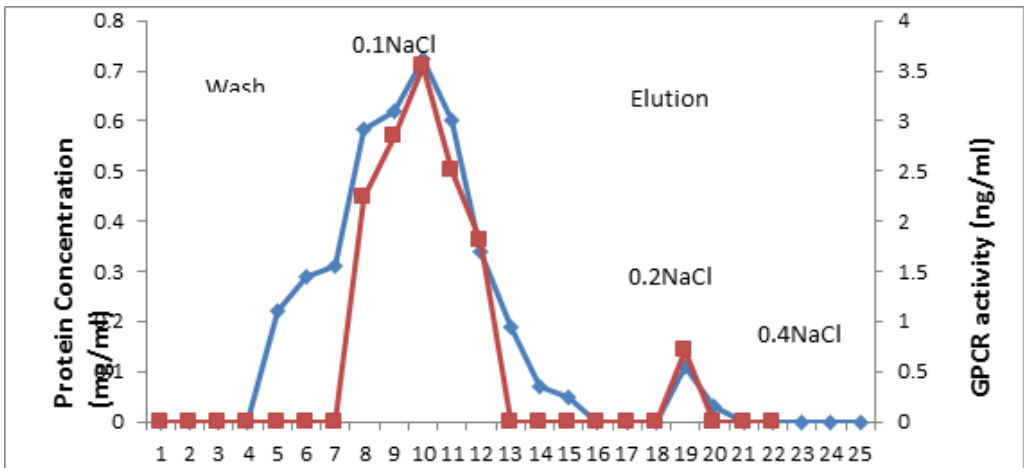
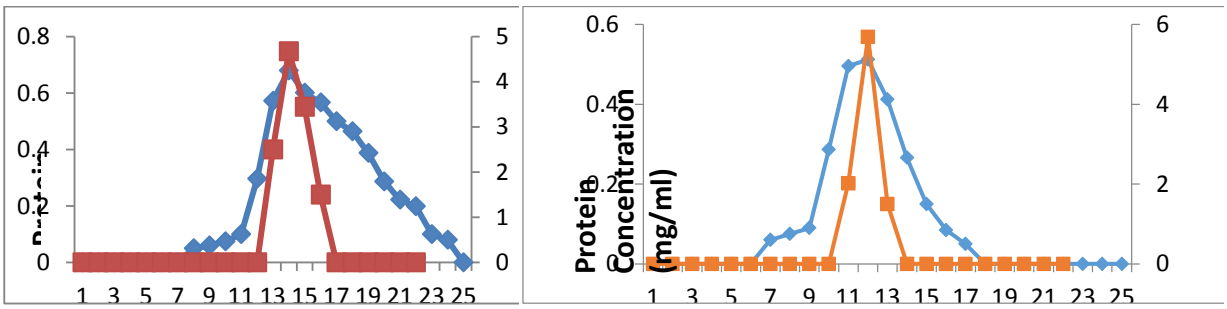


Figure (2) : Ion Exchange for G3

Gel filtration chromatography :

Results displayed in figures (3,4) which showed a single protein peak after eluted with elution buffer that was identical with the peak that presented GPCR concentrations at fractions number (14) for G1 and show of single active protein peak that was identical with the peak that presented GPCR concentrations at fractions number (12) for G2 which show a single active protein peak that was identical with the peak that presented GPCR concentrations at fractions number (12) for G3 .



A- Gel -filtration for G1 **B- Gel -filtration for G2**

Figure (3) : Gel -filtration for A-G1 B- G2

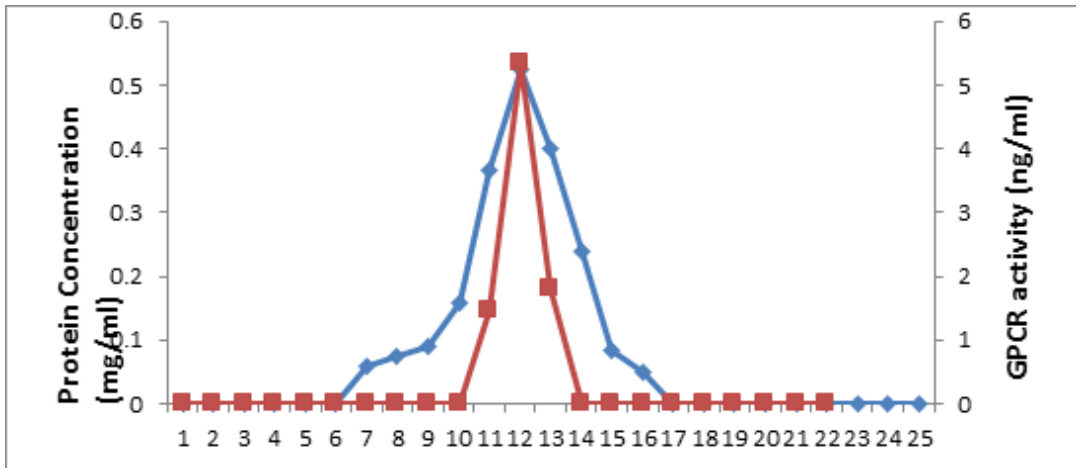


Figure (4) : Gel -filtration for G3

Table (2) : Volume , protein concentration , GPCR concentration , specific activity, purification fold for all purification steps of GPCR for G1,G2 and G3.

G1

Steps	Volume (ml)	Protein con.(mg/ml)	GPCR con. (ng/ml)	Specific activity (ng/mg)	Purification fold
Crude	30	0.89	6.61	7.42	1.0
Ion exchange	12	0.718	5.32	7.49	1.1
Gel filtration	8	0.32	3.26	10.18	1.37
G2					
Crude	30	0.92	6.19	6.72	1.0
Ion exchange	10	0.713	5.688	7.9	1.17
Gel filtration	8	0.512	4.89	9.55	1.42
G3					
Crude GPCR	30	0.94	5.322	5.66	1.0
Ion exchange	9	0.723	4.49	6.21	1.1
Gel filtration	7	0.525	3.52	6.7	1.18

Determination of purity and molecular weight of GPCR by SDS-PAGE:

The molecular weight and purity of GPCR that purified from G1,G2 and G3 were determined by sodium dodecyl sulfate –polyacrylamide gel electrophoresis (SDS-PAGE) ,as shown in figure(5) .

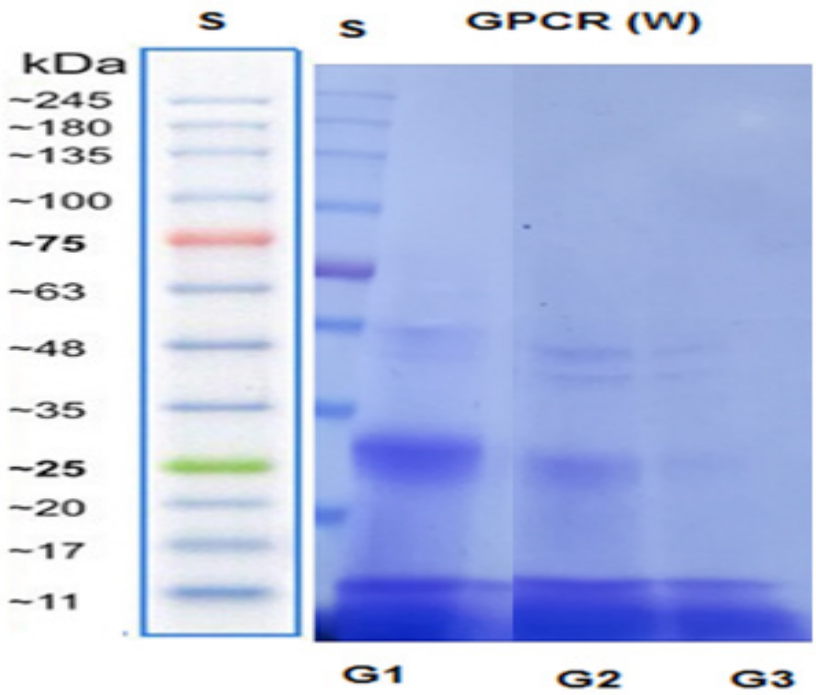


Figure (5) SDS-PAGE for purified GPCR in G1,G2 and G3

Results indicated the appearance of two bands of GPCR in G1at (~30 KD) and (~51 KD) . This could be due to the isoform of GPCR. As it was reported that the monomeric form is important to activate G-protein for binding , and they stated that further studies are required to understand the function of the dimerization of such receptor for its biological function [20].

Results ,also , showed the appearance of three bands in G2 that related to GPCR in (~ 29 KD) and (~ 47,49 KD) . In G3 the bands appeared similar to that in G2 but with highly clearance related to its highly concentration as show in table (2) .

The glucagon – like peptide -1 receptor (GLP1R)is a class B GPCR with molecular weight of 53 KD, that found in type two diabetes in previous study .According to the previous researches that improved molecular weight of GPCRs about ~30 and ~50 KD , such as GLP-1R (~53 KD), GPCR 40 (31.438), α_2- Adregeric receptor (~50 KD) ,β_2- Adregeric receptor (47.058KD) [21] . So, in this study may be one or more of the above receptors were purified in diabetic and diabetic nephropathy . further study is important to know amino acid sequences in the purified receptors to know the type of GPCR that purified in these patients exactly.

Conclusion

Conclusion that obtained from this study that 2 bands of GPCR appear in control group while there are three bands appeared in G2 and G3 with different concentrations which may be useful in early detection and active treatment to controlling diabetic and diabetic nephropathy patients .

Conflict of Interest : None

Funding-self or other source : Ibn-Al-Haitham College of Education for Pure Science / University of Baghdad

Ethical Clearance : All studies were conducted in accordance with the ethical committee of Ibn-Al-Haitham College of Education for Pure Science / University of Baghdad**.**

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The Role of Overexpression of Rab1A in Human Pancreatic Cancer and its Association with Poor Prognosis

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Abstract

Rab1 is a member of RAS oncogene family,its role ismediating vesicle transport between the endoplasmic reticulum (ER) and Golgi. Rab1A contributed to human prostate cancer,colorectal cancer (CRC), hepatocellular carcinoma(HCC), gliomasand tongue squamous carcinomas(TSCCs) tumor proliferation and migration.Because pancreatic carcinoma is one of the common human cancers with high lethal malignancy rate,we investigated the expression of Rab1A inpancreatic carcinoma.171 samples with normal pancreatic tissues and 176 samples of pancreatic cancer tissues were obtained and prepared atthe Pathology Clinic, Uppsala University Hospital (Uppsala, Sweden).This study was noticedthat Rab1A is overexpressed in pancreatic carcinoma tissues both in mRNA and protein levels. Moreover, survival analysis showed that Rab1Aoverexpression in human pancreatic carcinoma is associated withpoor prognosis ($p<0.05$). Strikingly,aberrantexpression of Rab1A in pancreatic carcinoma patients is associated with an elevated level of mTORC1 (p-S6K1) signaling. Our results demonstrate that Rab1A may have apotential role in pancreatic cancer progression in anmTORC1 dependent manner, and further emphasizes the role of Rab1A in human malignancies. Together, these results indicate that Rab1A overexpression couldpromote a new prognostic predictor for human pancreatic cancer.Thus, studying the regulation of Rab1A in pancreatic cancer is urgently needed.

Key words: *Rab1A, pancreatic cancer, prognostic marker, mTORC1*

Introduction

Pancreatic cancer is an aggressive and common leading cause of cancer related death after lung, breast or prostate and colorectal cancer worldwide.[1] Because the lack of the reliable diagnosis methods, the majority of patients are diagnosed too late with advanced disease. In general, there arefew curative options appreciably can change itsaggressivenessand lethality.[2,3] Thus, Survival rate with pancreatic cancer is fairly low, with one-year relative survival of less than 25%.[1,3] Genetic mutationsdrives pathogenesis in the pancreas with a total of 5%–10% of all pancreatic cancers.[4-6] Chronic pancreatitis seems to be associated with 4% of cancer cases. [7] Aside from mutations or pancreatitis, overexpression of certain oncogenes has been previously reported to promotethe development of human pancreatic cancer.[8] Becauseof the high lethality rate of pancreatic cancer, there is urgent need to identifynew target genes underlyingpancreatic cancer to improve treatment and survival.Rab1A is a member of RAS oncogene family which is highly conserved in mammals. It is a GTPase predominantly anchored to Golgi membrane and mediates ER to Golgi vesicular trafficking. [9]Beyond Rab1A role in vesicle trafficking, it has been found aberrant level ofRab1A act as anoncogenein colorectal cancer,which is associated with poor prognosis.[10]Overexpression of Rab1A has been linked to multiple human diseases particularlycancer including colorectal cancer (CRC) [10], hepatocellular carcinoma (HCC) [11], glioma[12], prostate cancer [13,14] tongue squamous carcinomas (TSCCs) [15] and cervical cancer.[16]Consistently with the role of ER-Golgi membrane system in cell signaling,Rab1A overexpression has been proposed to promote the pathogenesisof CRC and HCC by activating the mTORC1 signaling pathway.[10]mTORC1 is well known as a central regulator of cell growth and its elevated level

is associated with multiple human diseases particularly cancer.[10,17]The recent findings [10-16]indicate that Rab1A might be a new therapeutic target for cancer therapy. Despite the contributory role of Rab1A in different types of human malignancies,until now, Rab1A has not been deliberated in the context of pancreatic cancer studies. In this study, we investigated Rab1A expression in human primary tissues from pancreas adenocarcinoma, andassess the relationships between Rab1A expression and patient survival.

Material and Method

Patients and pancreatic tissue samples: 171 samples with normal pancreatic tissues and 176 samples of pancreatic cancer tissues were obtained from October 2015 to November 2018. The pancreatic tissue array was prepared by at the Pathology Clinic, Uppsala University Hospital (Uppsala, Sweden).The overall survival time was calculated from the day of therapyto the end of the follow-up or the date of death because of the recurrence andmetastasis. The median patients' follow-up was of 33 months (range: 2-80 months). Patients with low Rab1AmRNA expression were all alive while patients with high Rab1AmRNA expression were 84 alive and 92 dead.

Immunohistochemistry (IHC): The standard streptavidin-biotin-peroxidase complex method was used for IHC staining. Tissue sections were incubated with a Rab1A-specific antibody (Antibody HPA039442, Antibody CAB003838, Antibody CAB018346) or S6K1 specific antibody (Antibody HPA056141 andAntibody CAB005331). The intensity of the staining was calculated as follows: no staining (0 positive cells); weak staining (0-10% positive cells); intermediate staining (10-50%positive cells); high stainingin the tissue cells (50-100% positive cells).

Analysis of Rab1A mRNA and protein levels: The genomic data from The Human Protein Atlas databases (https://www.proteinatlas.org) was used to analyze Rab1A mRNA expression alteration and protein expression level in normal and primary pancreatic cancer tissues. The website (proteinatlas.xml.gz) was utilized to download data including protein expressionand RNA seq data.

RNA seq: The standard RNA-seq protocol was used for measuring mRNAquantity. In short,RNA was extracted from tissue samples using the RNeasy Mini Kit (Qiagen, Hilden, Germany).Samples of high-quality RNA were used in the sequencing.

Statistical Analysis: Survival analyses were carried outby Kaplan-Meier plots and the log-rank test. Results are considered significant when p value is less than 0.05.

Findings

Rab1A is overexpressed in human pancreatic tissues

Genomic findings from previous studies suggest that Rab1A gene acts as an oncogene and is amplified in many cancer cases. [1-4]Therefore this studyinvestigated Rab1A mRNA and protein expression in 176 pancreatic cancer samples and 171 normal pancreatic tissues available in The Human Protein Atlas database (https://www.proteinatlas.org).Analysis of the primary data shows Rab1A mRNA and protein levels are both higher in pancreatic cancer tissues compared to normal pancreatic tissues (Figure 1A, 1B and 1C). Since the increase in Rab1A occurs both at transcriptional and translational levels, this suggests Rab1A mRNA and proteinexpressions are positively correlated and that increased in Rab1A level is due to change in gene expression. Generally, our results indicate that high Rab1A expression level in pancreatic cancer resemble those seen in other studies with particularhuman tumors such as HCC and CRC. [10,11]Therefore,we think that the results of this study are useful for supporting evidence that may explain many of the findings related to the development of pancreatic cancer.

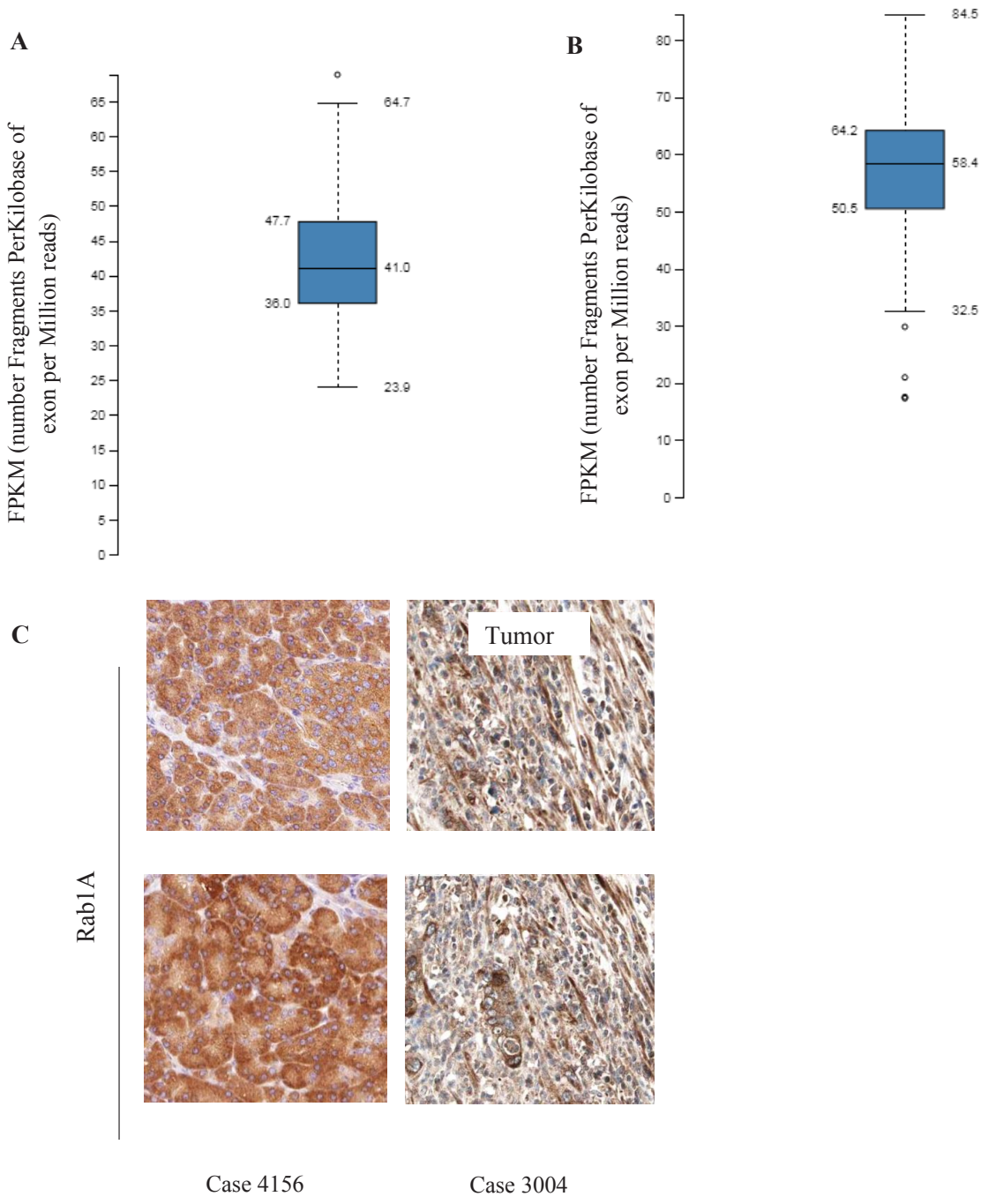

Figure 1: Rab1A is overexpressed in human pancreatic tissuesA.

RNA-seq data has been mapped usingthe number FragmentsPerKilobaseof exon per Million reads (FPKMs). Shown is mRNA level in 171 pancreatic normal tissues in The Human Protein Atlas database. (www.proteinatlas.org/ENSG00000138069-RAB1A/tissue/pancreas#cbox).**B.**RNA-seq data is proposed as average number Reads PerKilobase genemodel and Million mapped reads (FPKMs). Shown is mRNA level in pancreatic carcinoma 176 samples. Data is downloaded from The Human Protein Atlas database(www.proteinatlas.org/ENSG00000138069 RAB1A/pathology/tissue/pancreatic+cancer).**C.**IHC staining for the primary human pancreatic cancer and noncancerous tissuesin the The Human Protein Atlas database (www.proteinatlas.org/ENSG00000138069-RAB1A/tissue/pancreas#img) and (www.proteinatlas.

org/ENSG00000138069-RAB1A/pathology/tissue/pancreatic+cancer#img). Shown are representative images of Rab1A staining of tumor and non-cancerous tissue sections. Scale bar, 50 µm.

Rab1A overexpression is significantly associated with poor prognosis in pancreatic cancer patients

To evaluate the clinical significance of Rab1A overexpression in pancreatic cancer patients, we analyzed the relationship between Rab1A expression levels and patient survival.Kaplan-Meier survival analysis of pancreatic cancer patients was separated into two groups by the median value for Rab1A-positive staining then validated. Our analysis reveals that overall survival of pancreatic cancer patients with high Rab1A expression is significantly worse than those with low Rab1A expression in pancreatic patients (Figure2A). Thus, Rab1A level can provide predictive value for the outcome of pancreatic cancer patients and our observation resembles those seen in HCC and CRC. [10,11]Our analysis further supports the finding that Rab1A overexpression is significantly associated with an elevated risk of pancreatic cancer related death.These findings demonstrate that Rab1A is an independent prognostic signature of poor survival in pancreatic cancerpatients.

In support the task of high Rab1A expression in signaling pathways and oncogenesis[1-4], we investigated the level of P-S6K1, the effector of mTORC1, in pancreatic cancer patients. To investigate this phenomenon, we checked the P-S6K1protein levels of pancreatic cancer patients by using IHC primary data deposited in The Human Protein Atlas database. Protein primary data analysis reveals that P-S6K1 protein level is generally higher in pancreatic cancer tissues compared to normal pancreatic tissues (Figure 2B and 2C). Our results are useful as supporting evidence that may explain many of the findings related to the development of pancreatic cancer.Altogether these outcomesdemonstrate that enhancingmTORC1 signal may be crucial for Rab1A to advance the pathogenesis of pancreatic cancer and that Rab1A could be new guide for targeted cancer therapy as seen in other malignancies. [18]

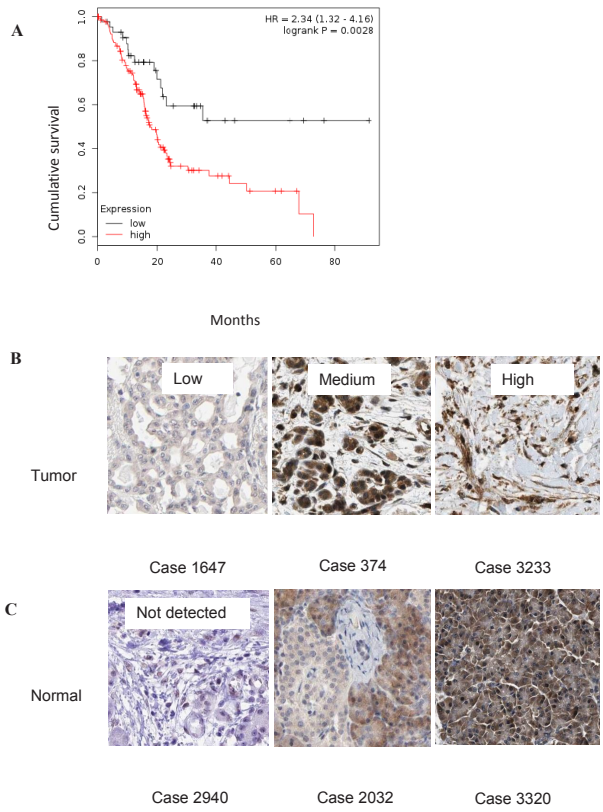

Figure 2: Overexpression of Rab1A in pancreatic cancer is associated with poor survivalA.

Kaplan-Meier survival analysis comparing the overall survival time of pancreatic cancer cases separated intotwo groups, low or high mRNA level of Rab1A. The p value was computed by log rank test.**B.**IHC staining for the primary human pancreatic cancer tissues in The Human Protein Atlas database (www.proteinatlas.org/ENSG00000108443-RPS6KB1/tissue/pancreas#img). Representatives are images of p-S6K1 staining of sections of tumor tissue. Scale bar, 50 µm.

C. IHC staining for the non-cancerous tissues in the The Human Protein Atlas database**(**www.proteinatlas.org/ENSG00000108443RPS6KB1/pathology/tissue/pancreatic+cancer#img). Shown are representative images of p-S6K1 staining of normal tissue sections. Scale bar, 50 µm.

Conclusion

Rab1 is aGTPase generally mediate vesicle shuttle betweenthe ER to Golgiapparatus.[19] Moreover, Rab1A involves in stimulating signaling pathways such as mTORC1 pathway.[10] To date, its role in human disease particularly oncogenesis remains unknown. Only until was Rab1Afound to be involved in oncogenic growth in many human cancer types. [1-3]Thepreviously mentioned

studies suggest that Rab1A could play important role in hyper-proliferative cancer.Sincepancreatic cancer is an aggressive disease, therefore we think that Rab1A could play important role ininitiation and development of pancreatic cancer.The present study finds that Rab1A is highly expressed in pancreatic cancer. These results further support the idea of that abnormal Rab1A expression could be a common phenomenon in certain human cancers.[11,20] Although our findingsdemonstrate that Rab1A is highly expressed in pancreatic tumors, the importance of elevation Rab1 expression in pancreatic cancer remained unclear.

Significantly, human pancreatic cancer patients with elevated Rab1A expression associate with poor survival. Log rank analysis demonstrates that mRNA level of Rab1A is anself-sufficient prognostic marker for pancreatic cancer patients.In contrast,low Rab1A expression associate withbettersurvival. These findings further support that Rab1 A hyperactivation can promote development of pancreatic cancer. [6]

mTORC1 is a principal regulator of signal transduction pathway in reaction to amino acid signals. [10] Recently, studies show amino acid signalsare capable of phosphorylatingS6K1, final effector target of mTORC1, in a mechanism is dependent on Rab1A.[10,11]Here we show that p-S6K1 is overexpressed in pancreatic cancer. This overexpression is closely correlated with Rab1 overexpression in primary pancreatic tissues. If that is true, these results may explain mechanisms of high Rab1A expression are in the cancer pathobiology.Thus, more studies need to be done to further investigate whether this common phenomenon drive oncogenic growth in pancreatic cancer. In addition, more analysis needs to be done to investigate whether Rab1A overexpression is correlated with pancreatic cancer sensitivity to rapamycin, mTORC1 inhibitor. Further investigation in this field could lead to a predictive marker for enhancing mTORC1-targeted therapy in pancreatic cancer.

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Ethical Clearance: Written informed Consent for the Ethical use of The Human Protein Atlasarchived data in our Research was obtainedfromthe administrative coordinator of The Human Protein Atlas research ethics committee(a Swedish-based program fundedfrom the Knut and Alice Wallenberg foundation).

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Prevalence of Urinary Tract Infections in Adult and Child Patients

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Abstract

Urine Samples collected from three hospitals in Baghdad city from adult and child patients suffering from urinary tract infections for 6 months. The samples get reached 1152 , the patients old were between few days to 80 years after urine cultured isolation and diagnosis of bacteria done with a modern methods *Escherichia coli* reached the highest ratio reached 36.36% , In adults between . 49.35-50% , In child 42.56% followed by *Klebsiella spp*. In ratio . 11.08% , 6.06-12.98 in adult and 10.49% in child, followed by *Proteus mirabilis* 8.86% where they ranged from 6.93-9.09% in adult to 9.91% in child . Also recorded others species from Enterobacteriace in lowest ratio where they ranged from 0.16% to 3.48% Also observed Gram positive bacteria in highest ratio *Streptococcus spp*. 7.43% followed by *Staphylococcus aureus* in 5.53% , *Enterococcus* in 3.48% and in lowest ration . Species from .*Staphylococcus* between 0.16% -0.79% .Also observed .*Candida spp* were about 3.32% in adult and child .*Proteus mirabilis* were Selected because there different complications in UTIs Patients specially Stone formation , obstructed the catheter, and renal damage. These bacteria were diagnosis in API 20 E and Vitek2 , sensitivity test its performed depended on gender and age of patients *p .mirabilis* the highest percentage in the age groups 31-40, 41-50 .yeras old , 18.75% 25.0% respectively in adult both sexes .while in child the highest percentage in the age groups 10 years old ,55.88% in male than female , also observed highest ratio adult patients with Urinary catheter who are in hospital many significant differences when used different antibiotics. There was a significant impactor effects , for Impenim(IMP) and Meropenemin both sexes in adult and child. Also seen adult samples sensitive for Gentamicin, Cefoxitin,

Aztreonam and Ticracillin highest in male than Female, the same results shown in child samples .In addition sensitivity to others antibiotics such as Levofloxacin, Ceftazidim, Cefepime.Inadult,female samples were sensitive to pipracillin,ceftriaxone and Cefotetan highest than male .Many , significant differences were observed for *P. mirabilis* resistant to different antibiotics such as Cefepime,Cefotaxime, ceftriaxone, pipracillin, Levofloxacin,nitrofurantion,Cotrimaxz in adult male highest than adult female.While in child samples were resistant to Meropenam , Naldalie acid, Trimethprim . nitrofurantoin ,cifxime, Ticaracillin and ceftriaxone. Highest in male than female and female child resistant to Sulfanethoxazonl , highest than male.

Keywords: *UTIs bacteria, Antibiotic , Proteus mirabilis and UTI.*

Introduction

Urinary tract is the one of most common area of bacterial infection caused UTI occurring from the neonate to the geriatric age groups encounters in medicals today , especially in females. In men are less common may be occur after 50 years of age [1][2]. UTIs caused by bacterial colonization of the urethra. Bacteria normally colonized the urethral opening then colonize the urine [3][4].The most prevalent agent for UTI ***Escherichia coli*** which caused 80-90% of UTIs in adults[5]Followed by ***Klebsiella*** spp. and ***Proteus mirabilis*** which frequently contributed with stone disease [6][7], and other different species of Gram positive and negative [8].Urine is normally a sterile fluid,UTIs are two typesUpper UTIs (U-UTIs) and Lower UTIs (L-UTIs) [9][4]. Occurrence of UTIs in adult males aged under 50 years is low, with adult women being 30 times more than men. In most

cases of UTIs bacteria is the most common causes,while some cases of UTIs by fungi and viruses are rare[2][10][11]. In children up to 7% of girls and 2% of boys will have asymptomatic UTI. [12]. The prevalence of urinary tract infection depended on age and sex of children [8]. In infants is greater than younger age, and with7% among newborns[13]. Most UTIs in children produced by ascending infections and the hematogenous spread may be more common in the first 12 week of life[14].The most commonest UTIs in children ***Escherichia coli***60-80% of cases ***Proteus spp***. (common in boys and in children with renal stones) ***Enterococcus , Klebsiella***, and Coagulase–negative Staphylococci [15].Risk factors for UTI in children is limited, and it associated with constipation,bladder instability,encopresis[16][17].Bathing and back to front wiping[14].Reflux of urine from the bladder up to the ureter, is common in children with anatomic abnormalities of the urinary tract and may be caused ascending infection and kidney damage [2].In UTIs , sometimes the urethral catheter a closed sterile system that is consist from tube inserted via urethra is used as urinary drainage [18].Many types of bacteria can be colonized this catheter and caused catheter associated urinary tract infections (CAUTIs)[19][20][21]. Microorganism can invade and cause UTI through three major routes: lymphatic pathways , ascending ,and hematogenous[22][23].Urinary catheterization and cystoscopy the mean instrument that cause of hospital acquired UTIs in both sexes[22][24]. Hematogenous or blood borne route spread occurs as a result of bacteremia[22].WHO report that UTI bacteria resistant for antibiotic[25]. *Proteus mirabilis* bacteria caused many complications such as renal stone , catheter obstruction ,kidney damage[26][27][28]. Objectives of the study investigation bacterial species that caused UTIs , isolation and diagnosis *P. mirabilis* that caused complicated UTIs, and study *P. mirabilis* sensitivity for different antibiotics .

Method

Urine samples collection

Urine samples(1152) were collected from three sites, for 6month,from patients suffered from UTIs,mid stream urine samples were collected in sterile containers[29]. sampleswere cultured on blood agar and MacConkey agar plates.Gram negative bacteria isolates were identified byA.Different standard biochemical : 1.API20 E system 2.IMVC test 3.TSI4.Gelatin liquefaction 5.VITEK2 system for *Proteus mirabilis* B. Oxidase test and pigment production for *Pseudomonas* spp.

Gram positive isolates were identified by:1. catalase test 2.coagulase test 3.mannitol salt agar *S.aureus* .Tests were done according to Collee *et al* and Realonds[30][31].

Antibiotic sensitivity test

56 samples of *Proteus mirabilis* cultured in Mullar-Hinton agar then different antibiotic disc as shown in Table1were placed on the plates .

Table1: List of antibiotics with its abbreviation

Antibiotics	Company	Origin	Abbreviation
Cefotetan 30 µg	Biotech	UK	CIN
Gentamicin 30 mcg	Biolab	Budapest	GN
Amikacin 30µg	Biotech	UK	AK
Cefepime 30µg	Biotech	UK	FEP
Cefoxitin 30µg	Biotech	UK	FOX
Cefotaxime 30µg	Biotech	UK	CTX
Ceftraxone 30 mcg	Biolab	Budapest	CTR or CRO
Chloramphenicol 30 µg	MAST	UK	C
Pipracillin 30 mcg	Biolab	Budapest	PIP
Ceftazidime 30µg	Biotech	UK	CAZ
Ciprofloxacin 5µg	Biotech	UK	CIP
Tetrcycline 30µg	Biotech	UK	T or Te
Aztreonam 30 µg	MAST	UK	AT
Levofloxacin 5µg	Biotech	UK	LE or LEV

Cont... **Table1: List of antibiotics with its abbreviation**

Meropenem 10 µg	MAST	UK	MRT or Mem
Tobramycin 10µg	MAST	UK	Tob
Nitorfurantoin 100 mcg	Biolab	Budapest	F
Impenim 10µg	Biotech	UK	IMP
Ticaracillin 25 mcg	Biolab	Budapest	TIC
Ampicillin 25 mcg	Biolab	Budapest	AMP
Azithromycin 15µg	Biotech	UK	AZM
Cefepime 30 µg	Biotech	UK	CFP
Trimethoprim Sulfanethoxazol 1.25L23.75µg	MAST	UK	STX
Nalidixic acid 30 µg	MAST	UK	NA
Nltrofurodontin 100 mcg	MAST	UK	NIT
Ceftazidime 30 mcg	Biolab	Budapest	CAZ
Trimethprim 15 mcg	Biolab	Budapest	Tm or TTR
Aztreonam 30 µg	MAST	UK	ATM
Cifxime 30 µg	Biotech	UK	CFM

Statistic Analysis

The statistic analysis system-SAS (2012) program was used to clarity the effect of difference factors in study parameters .Chi –square test was used to significant compare percentage.

Findings

1152patients suffering from UTIs distributed in both sexes and different age between few days to eighty years old .In the current study, *Escherichia coli* is the largest causes of UTIs in different olds (46.36%) distributed in Child ,42.56% -50% in adults followed by *Klebsiella spp*. 11.08% distributed in adults 6.08%_12.08% , and 10.49% in child.Followed by *Proteus mirabilis* 8.86% ,6.93-9.09% in adult and 9.91%in child .*Enterobacter spp.*distributed as 3.48-3.90% in adult and 4.08% in child *Acinetobacter spp.*1.37-3.48 in adult while 5.25% in child *Citrobacter spp.,Serratia spp.* Was the lowest percentage 0.16% in adult and child respectively all these species from Enterobacteriaceae family.*Pseudomonas aeruginosa*8.06% distributed 4.54%-8.06% in adult and 8.16% in child while *Pseudomonas fluorescens*0.16% in child only *Morganella ,Burkholdericepecia*in 0.16% in adult and child respectively .All these species as Gram negative bacteria also isolated Gram positive bacteria such as *Staphylococcus aureus*5.53% distributed in 1.51-1.73% in adults and 8.74% in child ,*Staphylococcus haemolyticus* 0.79% and *Staphylococcus xylosus*0.32%, *Staphylococcus saprophyticus 0.16%* all in child , *Streptococcus spp.* 7.43% as6.06%-8.66% in adult to 6.70% in child , *Enterococcus spp.* 3.48% as 4.08% in child only and the last causes of UTIs *Candida spp. 3.32%* distributed as 5.19 -7.58% in adult and 1.16% in child only as show in Figure (1,2,3,4) .

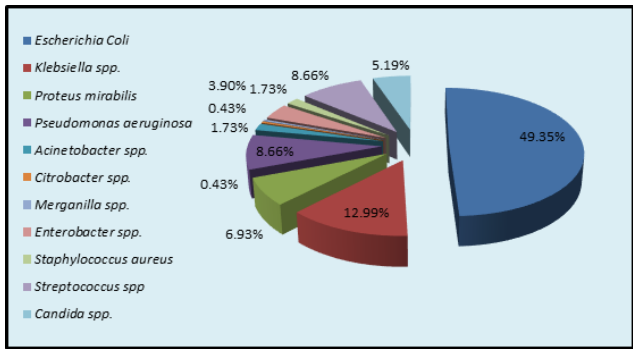

Figure (1) Distribution of bacterial specsies in three hospital isolated from UTIs

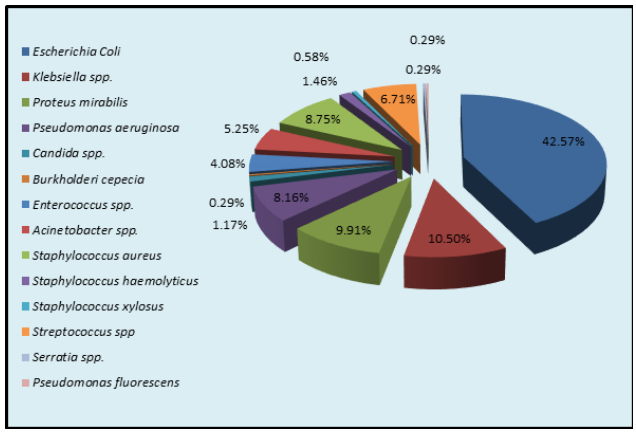

Figure (2) Distribution of bacterial species in Martyr Ghazi AL-Hriri hospital

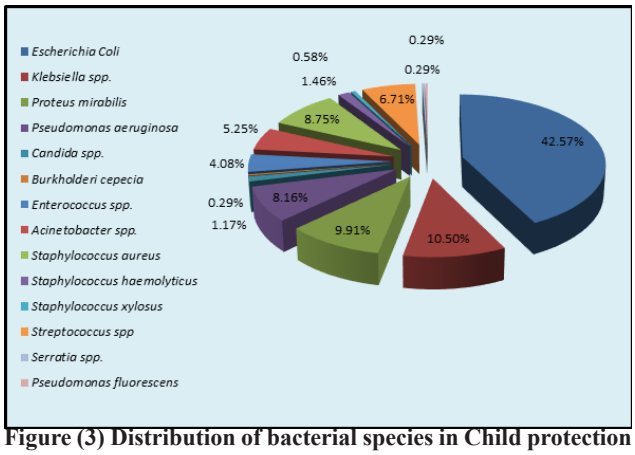

Figure (3) Distribution of bacterial species in Child protection hospital

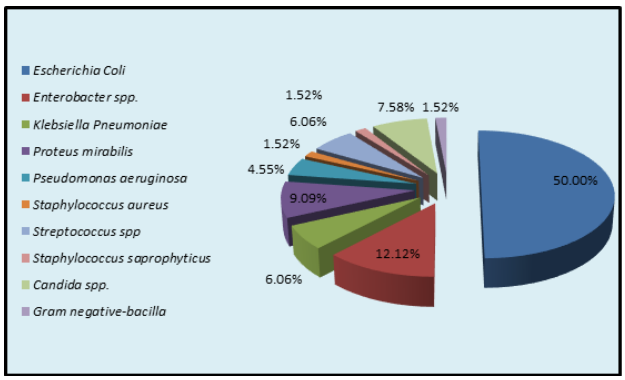

Figure (4) Distribution of bacterial species in Educational laboratoeries hospital

Proteus mirabilis 8.86% distributed in both sexes,the highest rate in adults age categories with highly significant value(**P<0.01) in 31-40 ,41-50 ,18.75% , 25.00%** the rate of infection in both sexes was equal , While in child the highest rate in age categories with highly significant value**(P<0.01) in 1 year – 10 year 55.88%**, the incidence of males was higher than females ,The highest rate with urinary catheter in **adult patients** than child.There are many significant differences**(P<0.01)**, **(P<0.05)**, when test different antibiotics depended on the gender and age, observed *proteus mirabilis* sensitive for Impenim, Meropenem. In both sexes to adult and child patients Also noted in adult patients *p. mirabilis* sensitive for Gentamicin ,Cefoxitin , Aztreonam,and Ticaracillin are higher in males than females . Moreover noted in child with same ratio for Gentamicin and Ticaracillin, Amikacin, Levofloxacin, Ceftazidim ,Ceftraxone ,and Cefepime. Also observed in adult females *p. mirabilis* sensitive for Pipracillin,Ceftraxone , Cefotetan higher than males.

Discussion

UTIs ,is one of common bacterial infection in adult and child in both sexes particularly in females than males[2][34]. Results of bacterial types infection in this study agree with WHO report for 2018 that which they pointed to increased the ratio of different types of bacteria to caused UTI complication and mortality vale of patients with UTIs[35].*Escherichia coli ,Klebsieella spp , Proteus mirabilis* respectively agreement with Ana et al[36]. This study were also agree with adult and with same the results of child and other Enterobacteaceae family members Karen[37].Gram-negative and Gram positive bacteria agreement with Wilson and Gaido[38]. The Recurrent UTI in different sexes specially pregnant women with higher percentage of *E. coli, P.mirabilis* and *klebsiella* than other women[39][40] . The study showed that child with highest ration UTI from adult specially in male than female in age **1-10 years** ,because male uncircumcised infants, anatomical abnormalities in urinary tract , poor toilet habits and hygiene[41] and obstructive anomalies , vesicoureteral reflux[42] .The similarities and differences in the type and distribution of uropathogens show a discrepancy from country to another due to many factors as environmental conditions , health practices , patient conditions, personal hygiene, number of patients examined, and laboratory procedures. *Proteus mirabilis* samples sensitive to Impenim (IMP) and Meropeneiem (MRT, MEM) in both sexes in adult and child , these results agreement with others study[43][44][45][46].*P. mirabilis* also sensitive indifferent degree to other antibiotic in adult male for Gentamicin , Aztreonam, cefoxitin, Ticracillin , Amikacin and more ever in child also sensitive to Levofloxacin Ceftazidim, Cefepime,In different levels most result agree with AL-Jmaily and Zgaer and Mahmoud et al[46][47].In this study adult female highest sensitive more than male to Pipracillin, Ceftraxone agreement with Mahmoud et al[47] and Cefotetan.There are many different Significant in adult male resistant for antibiotics in this study Cefepime , Cefotaxime , Ceftriaxone, Pipracillin, Levofloxacin , nitrofurantion, Cotrimaxzol, Chloramphenicol , In child in both sexes Meropenam, Naldalic acid Trimethoprim , nitrofurantoin, Cifxime,Ticaracillin and child female more resistant than male for Sulfamethoxazole .There are many study proved that development of resistant for antibiotics or other drugs from *P .mirabilis* characterized as plasmid-borne drug resistance.[48]

Conclusion

1.*E. coli, Klebsiella, P. mirabilis* the highest rate of G-ve bacteria .

2. *P. mirabilis* infected both sexes in different age.

3. *P. mirabilis* resistant to different antibiotics depended in sexes.

Conflict of Interest : Non conflict of interest with any side

Source of Funding : Self source

Ethical Clearance: Oral approval was taken from patients for samples collected

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Assessment the Health Awareness of Pregnant Women's Concerning Toxoplasmosis Infection

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Abstract

A descriptive study was conducted on pregnant women to assess their health awareness concerning toxoplasmosis infection. Randomized samples were selected (100) pregnant women during various trimesters of gestations attending MCHC. Questionnaire from was designed and consist socio-demographic and reproductive data, general information and questions related to samples awareness regarding the toxoplasmosis infection. The data were collected by using interview method and descriptive statistical procedures were analyze the data. The result of the study revealed that the highest percentage of the study sample were in age group 20-29 years, primary school graduate, house wife ,had no previous history to toxoplasmosis infection, had no close contact with cats, in third trimester of gestation, have more than two children and had no history of abortion.Regarding the health awareness of the study sample about their general information ,signs and symptoms, and effects of toxoplasmosis infection were below cutoff point which mean poor awareness while their awareness of concerning methods of transmission and preventive behaviors about toxoplasmosis were above cutoff point which mean good awareness. The study recommended for the importance of health education about toxoplasmosis risk factors to women of child bearing age.

Key words:*Pregnant women ;Toxoplasmosis infection ; Awareness*

Introduction

Toxoplasma gondii is one of the infectious agents of congenital TORCH infections cause's severe clinical outcomes in fetus and newborn[1]. Toxoplasmosis is a parasitic disease caused by toxoplasma gondii[2].The infection is characterized by non-specific symptoms with the consequent formation of cysts that may remain latent form in many organs[3].

There are four groups of individuals in whom the diagnosis of toxoplasmosis is most critical:-Pregnant woman who acquire their infection during gestation, fetus and new born who are congenitally infected, immune compromised patients, and these with chorioretinitis[3,4]. It is one of the most prevalent chronic infections affecting one third of the world's human population. The prevalence of toxoplasma gondii infection varies among different geographical regions [3].Up to half of the world's population is infected with toxoplasmosis .In the united states about 23% are affected and in some areas of the world this is up to 95% .about 200,000 cases of congenital toxoplasmosis occur a year[2].It is most common in Central America and central Africa, and much less common in the US. This variation can be explained partly by climate since temperature and humidity affect how long toxoplasma cysts remain infectious [5,6].

The infection in pregnant woman may cause devastating effects in the fetus. If the infected tissue of an animal being consumed it acts as a transmission mode of infection [1].Awareness and practice regarding this disease and preventive measures in life style for this parasitic infection should be given to the pregnant woman [1].Therefore, this study aimed to evaluate the level of awareness and practices on toxoplasmosis among pregnant woman who visiting the antenatal clinics or hospitals.

Objectives of the study:- To assess the health awareness of pregnant women's concerning toxoplasmosis infection**.**

Materials and Method

Design of the study:A descriptive study was carried

out to assess the health awareness of pregnant women regarding the toxoplasmosis infection.

Setting and sample of the study :The study was conducted of (100) pregnant women selected randomly during various trimesters of gestation visiting antenatal care center**.**

Tools of the study :The questionnaire was constructed for the purpose of the study through review of available literature and relevant studies. The questionnaire covered respondents general information , symptoms, transmission methods ,risks and effects on fetus and newborn baby and preventive behaviors regarding toxoplasma infection .

Data collection :The data were collected by using interview method and self – report techniques with study participants after obtaining permission from each of them.

Statistical data analysis:Methods were used in analyzing the data of the study were included the measurement of the following:-Frequency (F), Percentages (%) ,Mean of score and standard deviation, Rating and scoring of the scale**(**Yes) for correct answerscored (2),and (No) for incorrect answer scored(1)

Evaluate the awareness of the study sample according to the Cutoff point 2 +1÷ 2=1.5

Mean score above 1.5 means good awareness (adequate) and mean score below 1.5 means poor awareness (inadequate).

Findings and discussion

Table (1) Distribution of the study sample according to socio-demographic and reproductive characteristics N=100

Variables	%
Age group / years	22%
<20	39%
20 -29	30%
30-39	9%
>40	
Mean ± SD 27.1± 3.84	
Education level	
Illiterate	5%
Primary school graduate	32%
Intermediate school graduate	22%
Secondary school graduate	18%
University graduate and above	23%

Cont... **Table (1) Distribution of the study sample according to socio-demographic and reproductive characteristics N=100**

Occupation	
Worker	15%
House wife	85%
Previous history to disease	
yes	30%
No	70%
Close contact with cats	
yes	10%
No	90%
Duration of gestation	
1st trimester	34%
2nd trimester	27%
3rd trimester	39%
No. of children	
None	25%
1	28%
>2	47%
History of abortion	
Yes	39%
No	61%

Table (1) showed that the highest percentage 39% of the study sample were in age group 20**–**29 years, while the lowest percentage 9% of them their age were above 40 years and mean with standard deviation (27.1 ± 3.84), this result is agree with the finding of study conducted on pregnant women from Malaysia their findings reveals that the majority of Malaysian pregnant women were in age group of 20-29 years[1].Regarding the education level the results reveals to the highest percentage 32% of the Study sample were primary school graduate while the lowest percentage 5% of them were illiterate, the majority of them 85% were house wife , and 70% of the study sample had no previous history to toxoplasmosis infection and 90% of them had no close contact with cats. Previous study reported that level of education play an important role in preventing toxoplasma infection[1].

The same table showed that the highest percentage 39% of the study sample in their third trimester of gestation, 47% of them have two and more children and this result agree with other study who found that the most of the study sample were in their third trimester, also reported that the trimester of pregnancy and the number of children play an important role in preventing toxoplasma infection and this may be for increased women's awareness and continuous follow up[1,7], and 61% of the study sample had no history

of abortion ,while 39% of them had history of abortion our finding is supported by a previous study who stated that women who become infected while they are pregnant more exposed to abortion or still birth or the infection could spread to the baby and cause serious complications[8].

Table (2) Distribution of the study sample according to their awareness about general information of toxoplasmosis.

Items	Yes		No		Total	MS*
	F	%	F	%		
1-Have you read , heard or seen any information about toxoplasmosis	74	74%	26	26%	100	1.74
2-Have you ever been tested for toxoplasmosis?	34	34%	66	66%	100	1.34
3-Is toxoplasmosis caused by an Infection	33	33%	67	67%	100	1.33
4- Is toxoplasmosis caused by a poison?	15	15%	85	85%	100	1.15
5- Is toxoplasmosis caused by parasite?	55	55%	45	45%	100	1.55
Grand mean	1.42 (Inadequate)					

*MS= Mean of score

Table (2) demonstrated the grand mean of score (1.42) for women's awareness about the general information of toxoplasmosis were below cutoff point which mean poor awareness (inadequate). this finding is consistent with the previous study who founded that in the respondents answers on the general knowledge of toxoplasmosis ,majority of these pregnant women have no awareness or unsure about this parasitic infection[9].This finding indicates the importance of educating the pregnant women with the preventive measures [1].

Table (3) Distribution of the study sample according to their awareness about signs and symptoms of toxoplasmosis.

Items	Yes		No		Total	MS*
	F.	%	F.	%		
Can toxoplasmosis in pregnant women cause? 1-Fever and fatigue	38	38%	62	62%	100	1.38
2- Feeling like influenza (Headache, sneezing).	46	46%	54	54%	100	1.46
3-loss of appetite	41	41%	59	59%	100	1.41
4-Vomiting and diarrhea	44	44%	56	56%	100	1.44
5-Swollen glands	37	37%	63	63%	100	1.37
Grand mean	1.41 (Inadequate)					

*MS= Mean of score

Table (3) this table showed that the grand mean of score (1.41) for women's awareness about signs and symptoms of toxoplasmosis were below cutoff point which mean poor awareness (inadequate). Our finding is supported by a previous study which found that the most of pregnant women 83% were not sure about the risk factors and symptoms of toxoplasmosis and medical staffs had lack of knowledge on this parasitic infection so an appropriate health education could then be provided to pregnant women and the health care related staff to better understand manifestation of this parasitic infection [1,10].

Table (4) Distribution of the study sample according to their awareness concerning methods of transmission.

Items	Yes		No		Total	MS*
	F.	%	F.	%		
Can people get toxoplasmosis by 1-direct contact with cats feces	76	76%	22	22%	100	1.76
2-Cats eating infected rodents , birds or other small animals.	74	74%	24	24%	100	1.74
3-Eating under cooked meat.	52	52%	45	45%	100	1.52
4-Receiving blood transfusion	63	63%	35	35%	100	1.63
5-Ingestion of raw or partly cooked meat.	54	54%	46	46%	100	1.54
6-Un washed fruits or vegetables contaminated with infected cat feces.	77	77%	23	23%	100	1.77
7-Mouth touch after gardening without gloves.	78	78%	20	20%	100	1.78
8-Changing cat litter box.	82	82%	12	12%	100	1.82
9- Drinking untreated water and milk (un boiled).	33	33%	67	67%	100	1.33
10- Receives a transplanted organ.	35	35%	65	65%	100	1.35
Grand mean	1.62(Adequate)					

*MS= Mean of score

Table(4) this table demonstrated the grand mean of score (1.62) for women's awareness concerning methods of transmission were above the cutoff point which mean good awareness (adequate) ,other study showing that having a close contact with cats and cleaning their litter may transmit the disease to pregnant women [11], while the results of present study were disagree of the finding of previous study who founded that there was a low level of awareness about methods of transmission[1].

Table (5) Distribution of the study sample according to their awareness about the effects of toxoplasmosis.

Items	Yes		No		Total	MS*
	F.	%	F.	%		
1-Can toxoplasmosis passed from mother to fetus through placenta	64	64%	36	36%	100	1.64
2-Can toxoplasmosis cause congenital malformations	33	33%	67	67%	100	1.33
3- Can toxoplasmosis cause damaged the baby's eyes	38	38%	62	62%	100	1.38
4- Can toxoplasmosis cause brain and nervous system damage (seizures)	44	44%	56	56%	100	1.44
5- Can toxoplasmosis cause fetus death	48	48%	52	52%	100	1.48
6- Can toxoplasmosis cause miscarriage	75	75%	25	25%	100	1.75
7-Can new born baby born with no symptoms but the symptoms developed after that	28	28%	72	72%	100	1.28
8- Can pregnant woman develop serious complications after infection with toxoplasmosis	55	55%	45	45%	100	1.55
Grand mean	1.48(Inadequate)					

*MS= Mean of score

Table (5) this table demonstrated the grand mean of score (1.48) for women's awareness about the effects of toxoplasmosis were below cutoff point which mean poor awareness (inadequate).Some studies have been reports that exposure to infected cats by these women could lead to severe out comes to her carried fetus [11,12]. Other previous studies high light the importance of health education among the pregnant women to reduce the seroprevalence of this disease hence minimizing the adverse effects of infection in the fetus or newborn [10].

Table (6) Distribution of the study sample according to their awareness regarding preventive behaviors.

Items	Yes		No		**Total**	**MS***
	F.	**%**	**F.**	**%**		
Toxoplasmosis can be prevented by 1-Feeding your cat dry or commercial cat food and not letting it kill and eat rodents	75	75%	25	25%	100	1.75
2-Avoiding stray cats	85	85%	15	15%	100	1.85
3-Wear disposable gloves in changing cats litter box.	89	89%	11	11%	100	1.89
4-Making sure the cats litter box is changed daily.	88	88%	12	12%	100	1.88
5-Wash your hands after gardening	84	84%	16	16%	100	1.84
6- Washing all fruits and vegetables before eating	93	93%	7	7%	100	1.93

Cont... **Table (6) Distribution of the study sample according to their awareness regarding preventive behaviors.**

7-Cleaning all catting boards and utensils thoroughly after each use.	89	89%	11	11%	100	1.89
8-Wash your hands after handling raw meat.	84	84%	16	16%	100	1.84
9-Cook food to safe temperature.	85	85%	15	15%	100	1.85
Grand mean	1.86(Adequate)					

*MS= Mean of score

Table (6) this table demonstrate the grand mean of score (1.86) for women's awareness regarding preventive behaviors a bout toxoplasmosis were above cutoff point which mean good awareness (adequate). This finding is supported by a previous study in Malaysian and Thailand showed that pregnant women appear to have better precaution or preventive practices toward toxoplasmosis infection[13,14].Awareness about toxoplasmosis and its related preventive knowledge and behaviors may reduce the infection rate and its disease burden in pregnant women[1].

Conclusion

The study has concluded the following:-

The highest percentage of the study sample were in age group 20-29 years , primary school graduate , house wife , had no previous history to toxoplasmosis infection , had no close contact with cats , in third trimester of gestation , have more than two children and had no history of abortion

Regarding the health awareness of the study sample, the findings indicated that inadequate level of awareness about the general information, signs and symptoms, and effects of toxoplasmosis infection, while concerning methods of transmission and preventive behaviors about toxoplasmosis indicated adequate level of awareness.

Source of Funding :Self

Ethical Clearance :The data were collected from participants after obtaining permission from each of them.

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Cytotoxic Effect of *Vinca rosea* Aqueous Extracts on (L20B) Cell Line *In Vitro*

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Abstract

The present study investigated the cytotoxic effects of aqueous crude extracts of *Vinca rosea* leaves , flowers and seeds on cell line (L20B Cell line)*in vitro ,* by using double dilution series (concentration between 1.95 – 1000 µg/ ml.

The results showed that the cytotoxic effect of extract is dependent on type of extract, amount of dose and exposure time. The concentration 1000 µg/ml gave the highest growth inhibition (IR) (74 and 74%) to leaves and flowers respectively compared with control 100% after 24 hours exposure time, but seeds were (49%) after 48 hours. However, low concentrations of aqueous extracts were found to induced the L20B cells growth and proliferation (PR), which recorded (122, 123) % by treatment with flowers extract in 1.95 µg/ml after 72 hours. Crude aqueous extract of *Vinca rosea* had hormetic effect (Hormesis), because it also induced the proliferation of cancer cells by using low concentrations of the extract.

KeyWords: *L20B,* ***Vinca rosea****, cytotoxicity, Inhibitory Rate.*

Introduction

Cancer emerged as a leading cause of death in the world. It is a serious disease that kills millions of people every year, and during lifetime, it affects one in two men, one in three women, while it causes death of one in four women[1].Cancer remains a problem for scientists despite the existence of several methods of treatment. There are chemotherapy, physiotherapy and surgical therapies, but all of them were not convincing for doctors or the patient himself. Thus, research centers and researchers turned to find other alternative therapies for the existing treatments and took another approach that may have great hope to eliminate the disease. Commercial drugs cost large amounts to import, and their effectiveness is gradually lost by continuous use due to the resistance of cancer cells to these medicines [2]. Therefore, many countries worldwide paid much attention to their plants as the natural source of drugs [3]. The discovery of anticancer effect of alkaloids of *Vinca rosea* plant gave it a great medical importance, because these alkaloids are chemotherapeutical agents for different types of human cancers [4,5].

About 75 types of alkaloids have been discovered, some of which have anticancer effects, including vinblastine and vincristine [6], as well as the use of this plant for the treatment of diabetes [7]Several studies were also carried out to use the plant extract in the treatment of microbial diseases such as skin diseases[8].Mitosis inhibitors such as Vinca alkaloids, derived from the *Vinca rosea (Cathathanthus roseus)* occupy a special place among chemotherapeutical drugs used in the treatment of many types of cancer [4,9].In this sense, and in order to enhance the study of the effect of natural plant extracts found in the Iraqi environment against some cancerous and transformed cell lines as a first step to explore their counter effects, this study was designed (as a part of an extensive study of different cell types) to investigate the effect of crude extracts of the *Vinca rosea* plant in the local Iraqi environment in inhibiting the growth of the L20B mouse fibroblast cell line.

Materials and Method

• **Plant collection**: The *Vinca rosea* was collected as an ornamental plant in the gardens of the College of Education/University of Karbala, and the plant was classified by the Iraqi National Herbarium/

Public authority for the examination and certification of seeds of the Ministry of agriculture. After the plant was collected and cleaned, it was washed thoroughly by tap water, and the plant parts (leaves, flowers and seeds) were separated and left to dry in the dark and at room temperature in a well ventilated dry environment to prevent damage of the samples. After drying of these three parts, they were finely grinded with an electric mill, then preserved in clean plastic containers away from light, heat and moisture until use.

Preparation of crude aqueous extracts of *Vinca rosea*: The cold aqueous extract was prepared according to the method used by Harborne *et al*[10], by taking 50 g of the dry powder for each part of the plant and adding 250 ml of distilled water and left the mixture on the magnetic stirrer at room temperature for 3 days, then the mixture was filtered by gauze then by filter paper (Whatman No.1). After that, the supernatant was dried to get the dry powder from which the required concentrations were prepared.

Indicative chemical detection of effective compounds: The types of secondary chemical metabolites found in the studied plant extracts (alkaloids, terpenes, flavonoids and glycosides) were determined, depending on what is stated in [10].

Study toxic effects of *Vinca rosea* extracts on the growth of (L20B) cell line

Type of the studied cancer cells: The (L20B) mouse fibroblast cell line was used by passage (18). The cells were grown on tissue culture medium with Minimum Essential Media (MEM), supplied by Sigma (USA) company with 5% (Fetal Calf Serum / FCS) supplied by the company itself.

Cytotoxic effects: Multiple (96) tissue culture microtiter plates, flat bottom were used to perform this experiment, which included three stages:

Cell seeding:

- After the growth and multiplication of cells, the containers with monolayer were taken, then cells were harvested using Trypsin-Versin (T.V) solution.

- Twenty ml of the serum-containing culture medium was added to each container and mixed well. Then, the cells were counted by the (Haemocytometer) using the (1%) trypan blue dye according to what indicated by Freshney [11].

- By a micro pipette, (0.1) ml from the cell suspension was taken and placed in each well of the plate. Each well contained (1×10^4) cell / well. The surface of the plate was then covered with a special sterile transparent adhesive paper for this purpose and the plate was moved gently, after that incubated at ($37C^0$) until the next day to allow (cell attachment).

2- Exposure of cancer cells to the plant extract:

The next day of seeding, serial dilutions were done in sterilized test tubes for each type of plant extract using the MEM-Serum free media, and dilutions from 1/2 to (1/1024) started gradually, which yielded the concentrations from 1000 to 1.95 µg/ml) respectively, taking into account to prepare the dilutions simultaneously at work. The culture media was poured from the wells after lifting the adhesive paper. The column No. (1) was considered as a negative control, as 0.2 ml of the serum free culture medium was added to it, while to the columns from 2 to 12 the dilutions of the extract, which were prepared as (0.2 ml / well / concentration) were added, and then a new layer of adhesive paper was re-placed on the surface of the plate.

The plates were incubated at (37C), while exposure times were (24,48 and 72) per hour.

Cytotoxicity assay:

Crystal violet stain was used to detect the cytotoxic effect of the extracts on cells pursuant to the following:

After the end of each incubation period, the plates were taken and their contents were poured and then washed with Phosphate Buffer Saline (PBS) solution, and 0.1 ml of crystal violet stain.

was added to each well, and left for (20) minutes. The cells were then washed with PBS solution several times until the excess stain disappeared. After the plates were dried completely, the results were read using the ELISA microplate spectrophotometer at a wavelength of 492 nanometers. The mean inhibitory Rate / I.R was measured according to the equation indicated by [12], and the mean proliferation rate / PR was calculated according to[13].

Finding

Indicative detection of active chemical

compounds: Detection of chemical compounds in the *Vinca rosea* extract showed that they contained (alkaloids, terpenes, flavonoids and glycosides)**.**

Cytotoxic effect of *Vinca rosea* extract on (L20B) Cell Line

Cytotoxic effect of the leaf extract: The toxic effect of leaf extract was studied in the L20B cell line by passage (18). The results shown in figure (1), revealed that the inhibitory toxicity effect was on the first day of exposure because it gave the best results, when the highest percentage of inhibitory rate (IR) growth inhibition was 74% at the concentration of 1000 µg/ ml of the extract after the first 24 hours of exposure. After that, the inhibition decreases as the concentration decrease, however, it remains effective. The lowest concentration used of 1.95 µg / ml gave a 41% growth inhibition. It is also noticed that the inhibitory effect 48 to 72 hours after treatment with plant extract was generally similar to the treatment after 24 hours, although it began to decline over time, because the highest rate of growth inhibition was 61% and 40% (with a viability rate of 39% and 60%) at 1000 µg/ml concentration after 48 and 72 hours of treatment respectively.

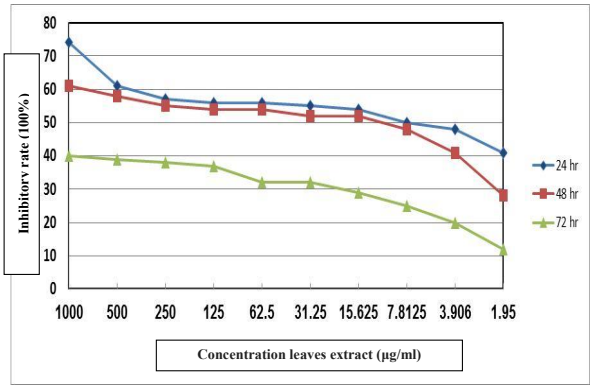

Figure (1): Effect of aqueous extract of the *Vinca rosea* leaves on the percentage of inhibitory rate on L20B cell line after different exposure time.

Cytotoxic effect of the flower extract: The results of the treatment of the L20B cell line with the *Vinca rosea* flower extract revealed that the inhibitory effect was similar for the three periods of time and gave a similar growth inhibition rates (IR) when using the first three high concentrations, reaching (74, 74, 65)% after 24 hours and (74, 73, 58)% after 48 hours and (71,62,56)% after 72 hours at the concentrations (1000, 500 , 250) µg / ml respectively. After that, the inhibition gradually began to decline with decreasing the concentration reaching to (8.3)% at 1.95 µg/ ml after 24 and 48 hours of exposure respectively as shown in figure (2). While the rate of cell growth was gradually increased beginning from 62.6 µg / ml making the proliferation rate reach to 123% at 1.95 µg / ml after 72 hours of treatment compared with control (100%).

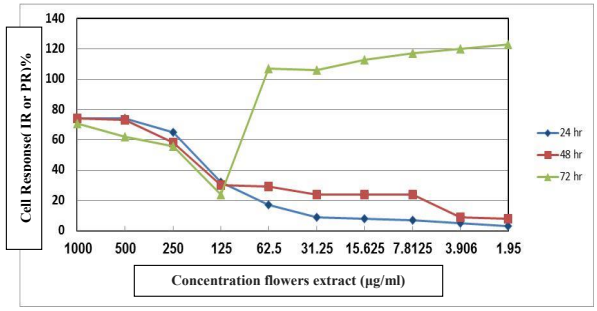

Figure (2): Effect of aqueous extract of the *Vinca rosea* flowers on the percentage of inhibitory rate onL20B cell line after different exposure time.

Cytotoxic effect of seed extract: There was less inhibition effect of the extract of *Vinca rosea* seeds on the L20B cell line compared with the two previous extracts (leaves and flowers). The best results were observed after 48 hours of exposure and then the rate of inhibition of growth decreased after 72 hours (increased cell viability). The toxic effect reached to 42% (cell viability 58%) after 24 hours of treatment at the highest used concentration of 1000 µg/ ml. In addition, the growth-stimulating effect appeared after 72 hours of treatment only, ranging from (110-122)%, and began to appear at the concentration of 7.8125 µg/ ml reaching to a minimal concentration of 1.95 µg/ ml respectively. figure (3).

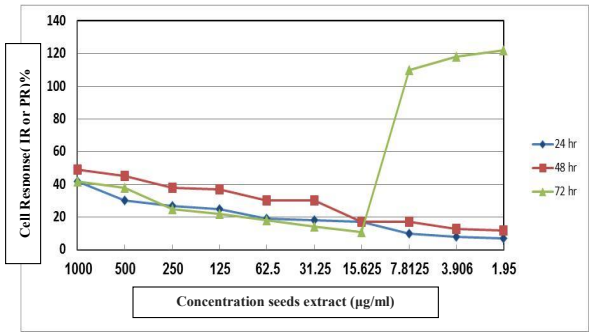

Figure (3): Effect of aqueous extract of the *Vinca rosea* seeds on the percentage of inhibitory rate onL20B cell line after different exposure time.

Comparison effect of the exposure time on the viability of the L20B cell line of the three studied extracts.

When a comparison between the viability of the three studied extracts (leaves, flowers and seeds) at each time of exposure, it was found that the first 24 hours of treatment showed the highest growth inhibition rates, as the viability of the cells was (26)%, (growth inhibition rate was 74%) for leaf and flower extracts and at the highest treated concentrations with 1000 µg/ ml (Fig. 4). In addition, results of 48 hour exposure were generally different from the 24 hour exposure, since the inhibition rate of leaves and flowers declined, while the highest rate appeared when the seed extract was used reaching to (49%), (51% viability) at 1000 µg/ ml concentration, and these rates decreased for all used concentrations compared with the exposure time of 24 hours (figure 5). The cell inhibitory rate within 72 hours of exposure was (40%, 71% and 42%) at 1000 µg/ml, (the viability rate was 60%, 29% and 58%) respectively, compared to control 100% (figure 6). It is observed that the viability rate of these cells begin to increase when low concentrations of flower and seed extracts are used to reach the highest viability rate (122 and 123)% respectively at 1.95 µg/ ml concentration. The results obtained showed that L20B cells were more sensitive to leaf and flower extracts and less sensitive to seed extract due to the high rate of cell viability.

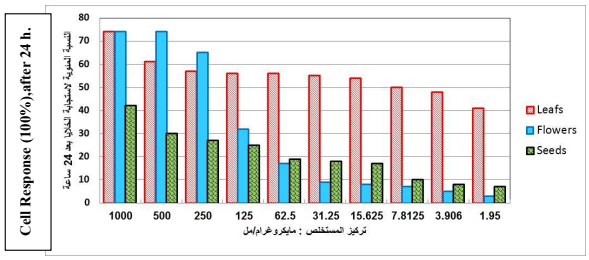

Figure (4): Comparison the effect of aqueous extracts of *Vinca rosea* on L20B cell viability after 24 h. exposure time.

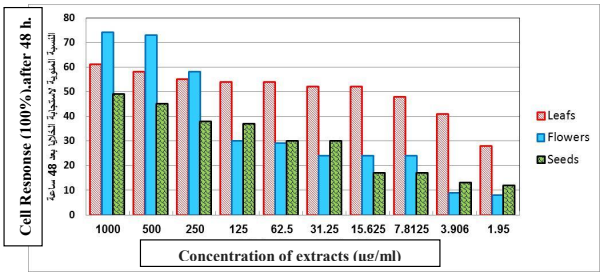

Figure (5): Comparison the effect of aqueous extracts of *Vinca rosea* on L20B cell viability after 48 h. exposure time.

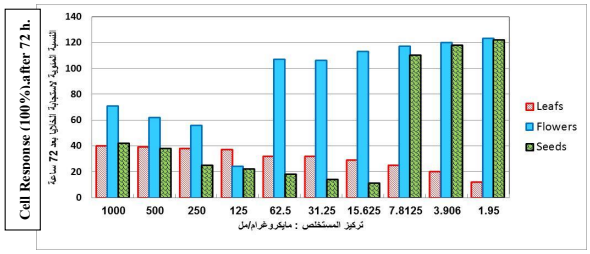

Figure (6): Comparison the effect of aqueous extracts of *Vinca rosea* on L20B cell viability after 72 h. exposure time.

Due to the importance of finding effective substances against cancer and finding more types of plants that possess these substances, the *Vinca rosea*, one of the locally available medicinal plants with different therapeutic properties, was [selected to identify the effects of crud aqueous extracts on L20B cell line] (as part of an extensive study in this field in many cancer cell lines) and the extent to which these extracts can be used as anticancer medical treatment in the future.The extracts of *Vinca rosea* contain many compounds and, as shown in the indicative chemical detection, the presence of alkaloids, terpenes, flavonoids and glycosides which may contribute to the better killing of cancer cells as a result of synergistic effect between them, which may reduce the toxicity of the used pure compounds.

The results showed that the crud extracts played a role in killing LB20 cells and inhibiting their growth and their division *in vitro*. The results indicated that the toxic effect of *Vinca rosea* on L20B cells was based mainly on the concentration used, exposure time and type of extract. The leaves extract had the best effect. On the other hand, it is observed that this type of cell, according to the results of this study, is sensitive to the prepared therapeutic aqueous extracts, as it inhibited the growth of cells, on contrary to the seed extract, which did not exceed the percentage of 49% inhibition after 48 hours of treatment using the high concentration of 1000 µg/ml, then the inhibitory effect is reduced when the exposure time is increased for all types of extracts. These results indicate that the effect of leaves and flower extracts is during the first 24 hours of exposure and at high concentrations, so when its effect is diminished, the living cells begin to reactivate and divide, showing the importance of giving repeated and continuous high doses to ensure the killing of all remaining cancer cells. The same thing occurs with the seed extract, which gives the best results after 48 hours of exposure and then the inhibition decreases and the cells reactivate.

A study performed by Yaseen *et al*[14]who compared between the two types of alcohol and aqueous extracts, and found that alcohol extracts are more effective than their aqueous counterparts on Hep-2 cells. This may be due to the fact that the ratio of the active substance extracted with ethanol (70%) is greater when using the aqueous extract, and this is reported by Harborne *et al*[10]. In two local studies on the same extracts in two cancer cell types - human cervical cancer cells (Hela-cells) [15] and human brain cancer cells (AMAG) [16], the results showed that both types of cancer cell lines were resistant to the aqueous *Vinca rosea* extracts (Leaves, flowers and seed), where low inhibition rates were recorded (not exceeding 46% in Hela cells and 64% in AMAG cells) using the highest concentrations (1000 µg/ml), while the L20B cells were shown to be sensitive to the same extracts. This is likely due to different receptors and antigenic determinants on the surface of each of the cancer and transformed cells.

The crud extracts of *Vinca rosea* contain a high percentage of alkaloids, which contain more than 75 types [17], as well as the presence of terpenes, phenols [18] and many mineral elements [19]. The proportion of secondary metabolic products in the plant varies according to the type of plant organ (leaves, flowers or seeds), and this is also affected by surrounding environmental factors[18]. Alkaloids are themost important and most effective substances in these extracts. The mechanism of their action is to inhibit the mitotic division, to keep the cells in the metaphase by inhibiting the polymerization of the protein Tubulin which is responsible for the formation of spindle fibers [9,20]. In addition, alkaloids inhibit the building of nucleic acids *in vitro*[21]. Several previous studies have also indicated that *Vinca rosea* alkaloids are effective against cancer cells, including human cervical cancer cells (Hela cells), because low concentrations cause inhibition to spindle fibers action [22,23]. On the other hand, Parekh and Simpkins [24] confirmed that these alkaloids affect on cancerous lymphocyte cells of rat and on human ovarian cancer cell line that are resistant to commonlychemotherapies used such as Cisplatin, as well as its being more effective than Taxol and Adriamycin.

Regarding the effectiveness of phenolic compounds, including flavonoids, which have an antioxidant effect by removing the generated free radicals and they direct the cell to enter apoptosis stage [25].

Many effective compounds effect on opposite directions depending on the concentration used. As noted by the above results, high concentrations inhibited the growth of L20B cells, while the low concentrations stimulated growth of these cells, increasing the viability by (122-123)% compared to the control (100%), indicating that the extract under study has a Biphasic effect [26], or Hormetic effect. There are many chemical therapeutical compounds, antibiotics, and toxins whose action is governed by the Hormesis phenomenon (abiological phenomenon common in toxicology), which act at low concentrations to stimulate, and may be useful to the organism, especially when the immune cells are activated, while high doses cause partial or total inhibition to the cells [27].

It is worth mentioning that the extract used in this study is crud extract, it contains many types of active compounds whose effectiveness was previously indicated or not mentioned, which supports the results of the emergence of antagonism in the influence on L20B cells depending on the concentration used. It is likely that its effect on the genetic material is in two directions, the first causes the inhibition of certain genes, while the other stimulates growth and multiplication.

Conclusion

The results showed that L20B cells were more sensitive to leaf and flower extracts and less sensitive to seed.

Conflict of Interest: Non

Funding: Self

Ethical Clearance: Non

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Detection of Human Papillomavirus in Cervical Mucus of Women with Spontaneous Abortion by Real-Time PCR

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Abstract

The aim of the study was to detect of human papillomavirus (HPV) in cervical mucus of women with spontaneous abortion by real-time PCR. Cervical mucus of 200 women with spontaneous abortion and 100 full term pregnant women were included in this cross-sectional which carried out in Kirkuk city-Iraq from beginning of 1st of October 2018 to the end of December 2018. HPV DNA was detected real-time PCR of genotype 16, 18 and 58 in cervical samples. The study showed that the highest rate of HPV DNA (29.5%) was detected in women with abortion comparing with pregnant women (12%) with highly significant relation of HPV with abortion (P. value: <0.05). The study stated that the highest rate of aborted women was infected with HPV 16 (37.29%) followed by HPV 58 (30.26%) while HPV 18 was found in 12.26% of those women and 13.56% of them was infected with both HPV 16 and HPV 58 genotypes. The study exhibited that 36.37% of aborted women with one time in their marriage age was infected with HPV followed by 28.995 in women with 2-3 time, the study presented that 52.5% of women with abortion was belonged to the age group 27-36 years.

Conclusion: Spontaneous abortion was highly associated with HPV infection specially with HPV 16 genotype.

Keywords: *Abortion; HPV; cervical mucus; real-time PCR*

Introduction

Recurrent miscarriage (RM) is defined as the occurrence of three or more consecutive losses of pregnancy. According to this definition, it affects about 1% of couples trying to have a baby [(1)]. However, many clinicians define RM as two or more losses; this increases the percentage of RM from 1% to 5% of all couples trying to conceive [(2)]. The role of infectious diseases in recurrent miscarriage is not clarified yet, but proposed an incidence of 0.5–5% [(3)]. There are some candidate infectious diseases such as *Listeria monocytogenes*, *Toxoplasma gondii*, rubella, herpes simplex virus (HSV), measles, cytomegalovirus, and coxsackie viruses. Infectious diseases may cause pregnancy loss by the following mechanisms such as direct infection of the uterus, fetus, or placenta, placental insufficiency, chronic endometritis, endocervicitis, amnionitis, or intrauterine miscellaneous infections[(4)]. Human papillomavirus (HPV) is one of the most prevalent sexually transmitted viral infections in men and women worldwide [(5)]. Human papilloma virus is a group of more than 200 related viruses, some of which are spread through vaginal, anal, or oral sex. Sexually transmitted human papilloma virus types fall into two groups, low risk and high risk[(3)]. Low-risk HPVs mostly cause no disease. However, a few low-risk HPV types can cause warts on or around the genitals, anus, mouth, or throat. In rare cases, they can cause recurrent respiratory papillomatosis, a condition in which benign tumors grow in the respiratory tract[(6)]. High-risk human papilloma virus can cause several types of cancer. There are about 14 high-risk human papilloma virus types. Two of these, HPV16 and HPV18, are responsible for most HPV-related cancers. The existence of more than 180 HPV genotypes has been reported [(7)]. According to the basis of oncogenic potential, HPV can be divided into 2 different groups-high-risk HPV (HR-HPV) and low-risk HPV (LR-HPV).

Generally, the HR-HPV included HVP16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66, and HPV68 [8]. It is well established that infections with HR-HPV can lead to cervical cancer. Moreover, recent evidence has shown that HPV infection is associated with the risk of colorectal, head, and oropharyngeal carcinomas [9]. To date, a succession of studies has been published to show the association between HPV infection and adverse pregnancy outcomes[6]. The aim of the study was to detect of human papillomavirus (HPV) in cervical mucus of women with spontaneous abortion

Methodology

A cross-sectional study was carried out in Kirkuk city-Iraq from beginning of 1st of October 2018 to the end of December 2018, including 200 women who attended to Kirkuk general hospital for curettage, and 100 controls (pregnant women at term of delivery) to find out DNA of HPV of HPV 16, 18 and 58 in cervical samples by real-time PCR (using Sacace biotechnology, HPV High Risk Screen Real-TM Quant). Women with positive TORCH serology IgM tests (against *T. gondii*, CMV, HSV) were excluded from the study. Cases were defined as women experiencing spontaneous abortion up to 20 weeks of gestation, who attended for medical attention and/or a curettage procedure. Controls were defined as women attending for delivery at term, with viable products.

Cervical swabs:

- Remove excess mucus from the cervical os and surrounding ectocervix using a cotton or polyester swab. Discard this swab.

- Insert the Sampling Cervical Brush 1.0-1.5 centimeters into the cervical os until the largest bristles touch the ectocervix. Do not insert brush completely into the cervical canal. Rotate brush 3 full turns in a counterclockwise direction, remove from the canal.

- Insert brush into the nuclease-free 2,0 ml tube with 0,3 mL of Transport medium (Sacace). Vigorously agitate brush in medium for 15-20 sec.

- Snap off shaft at scored line, leaving brush end inside tube, DNA were extracted and PCR was performed on all samples according to manufacture instructions. HPV DNAA is extracted from using RT-amplification and detected using fluorescent reporter dye probes specific for HPV or HPV IC. The HPV IC is an internal control and represents recombinant RNA-containing-structure which carried through all steps of analysis from nucleic acid extraction to PCR amplification-detection. The total reaction volume was 25 µl, the volume of RNA sample was 12.5 µl.

1. The reagents were thawed, and the tubes were vortexed and centrifuged briefly.

2. Requested quantity of reaction tubes were prepared including 3 extraction controls, negative amplification control and 4 standards.

3. The entire contents of the tube with RT-PCR-mix-2-FRT was added to the tube with DTT, thoroughly vortexed.

4. Tubes for samples, controls and standards were prepared. The results are interpreted by the presence (or absence) of the intercept between the fluorescence curve and the threshold line which determines the presence (or absence) of the Ct values for the sample. Based on the Ct values and on the specified values of the calibrators, QS1 HPV and QS2 HPV, the calibration line will give the values for the number of HCV cDNA copies (JOE channel) and for the number of internal control (FAM channel) in a PCR sample.

Statistical Analysis

Computerized statistically analysis was performed using IBM SPSS V23.0.0 statistic program. Comparison was carried out using; Chi square and T-Test.

Finding

The study showed that the highest rate of HPV DNA (29.5%) was detected in women with abortion comparing with pregnant women (12%) with highly significant relation of HPV with abortion (P. value: <0.05), Table 1.

Table 1: Detection of HPV DNA in mucus of cervix of aborted and pregnant women

HPV result	Aborted Women		Control group (Full term pregnant women)	
	No.	%	No.	%
HPV +ve	59	29.5	12	12
HPV -ve	141	70.5	88	88
Total	200	100	100	100

P. value: <0.05.

The study stated that the highest rate of aborted women was infected with HPV 16 (37.29%) followed by HPV 58 (30.26%) while HPV 18 was found in 12.26% of those women and 13.56% of them was coinfected with both HPV 16 and HPV 58 genotypes, Table 2.

Table 2: Coinfection between HPV genotypes (16,18 and 58) in women with abortion

HPV genotype	Aborted Women	
	No.	%
HPV16	22	37.29
HPV 18	9	12.26
HPV 58	18	30.51
HPV 16/18	1	1.69
HPV 16/58	8	13.56
HPV 16/18/58	1	1.69
Total	59	100

The current study exhibited that arte of 36.37% of admitted women who aborted one time in their marriage age was infected with human papilloma virus followed by 28.99% in women with 2-3 time of abortion with infection of human papilloma virus, Table 3.

Table 3: Distribution of HPV infection according to number of abortion

Number of previous abortion	Total No. (200)	Aborted Women	
		No (59).	%
1	88	32	36.37
2-3	69	20	28.99
More than 3	43	7	12.28

The study presented that 52.5% of women with abortion was belonged to the age group 27-36 years

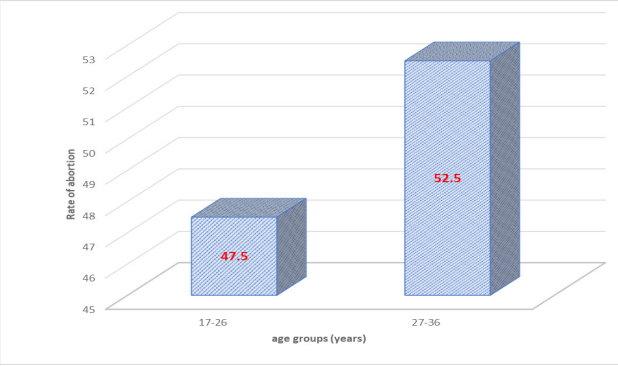

Figure 1: Distribution of women with abortion according to age

Discussion

It is important to study human papilloma virus detection in pregnancy, which represents a special immunological state that may be a risk factor for HPV infection [6]. Some studies have shown a higher frequency of human papilloma virus cervical infection in pregnant women compared to non-pregnant controls, possibly because of an effect of elevated estrogens which may affect the viral replication, or due to the altered immunity [7-9].

Human papilloma virus infection is common in the general population, including normal pregnant women. In normal full-term pregnancy, prevalence of HPV was reported to vary between 2.2 and 75% in the cervical tissue, with a summary estimate of 17.5% [5]. Correspondingly, the prevalence of human papilloma virus was significantly higher in the cervix and placental and aborted tissue of women who underwent spontaneous abortions [8]. Although the prevalence of HPV was significantly different in normal pregnancies and the spontaneous abortion group, our results based on 5 cohort studies and 3 case-control studies indicated that there was no significant association between human papilloma virus infection and spontaneous abortion. When the included studies reported HR-HPV infection alone. However, the pooled OR of 4 cohort/case-control studies [9-11] reported that the HR/LR–HPV infection indicated that HPV infection was a risk factor for spontaneous abortion. It was reported that there are 2 different pathways in the natural history of HPV infections, namely, the infectious virion producing pathway and the clonal transforming pathway [10]. The infectious virion producing pathway can lead to subfertility or early abortion and it is infectious. Oncogenic (HR) HPV types induce more rapid cell division arrest than LR-HPV or intermediate HR-HPV types. It takes the embryo longer to die after LR-HPV infection, which makes it possible to measure the spontaneous abortion [12].

In China, a 10.2% HPV prevalence was reported, with genotypes 16 and 58 being the most frequent (29% and 19% respectively) [13]. In the United States human papilloma virus prevalence reported was 35.6%, (11.6% low risk and 29.5% high risk types) [14]. Another study in the United States reported 29% prevalence, the most frequent genotypes being HPV16 (21%), HPV31 (12.7%), HPV18 (9%) and HPV51 (9%) and HPV6/11 (6%) [11]. In Spain, HPV prevalence found was 6.5%,

with HPV16 and HPV6/11 being the most frequent genotypes [15].

Coinfection with multiple HPV types has been associated with a higher risk of cervical abnormalities [16,17]. Longitudinal studies are needed in order to determine the risk associated with multiple infections in obstetric patients. Of relevance are the high risk genotypes, which may clear spontaneously after the inmmunological state is restored, or persist and may cause lesions. Also of importance are genotypes 6 and 11 identified in the mothers: if they are transmitted they could represent a risk for the newborn, because of the possible of development of laryngeal and respiratory papillomatosis later in childhood. The most common causes of pregnancy loss in the first trimester are of genetic origin [6]. Amongst other important risk factors are maternal age younger than 20 or older than 35 years old, placental inflammation and infection, but the etiology is often uncertain [5]. Conde-Ferráez *et al* [18] demonstrated that spontaneous abortion was associated to a previous pregnancy loss and to women's age older than 35 years old.

Conclusion

Spontaneous abortion was associated with HPV infection specially with HPV 16 genotype.

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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The Role of Proinfalmatory Cytokines (Interleukin-1 Beta and 6) in Pathogenesis of Breast Cancer

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Abstract

A cross-sectional study was carried out in Kirkuk city from of November 2017 to March 2018. The number of breast cancer women under study were 55 women whose ages were between 30-70 years old. These patients admitted to Kirkuk oncology center. The control group who were matched to the breast cancer patients studied, included 35 unaffected women and their ages were between 30-75 years old. These women presented Kirkuk General Hospital Four ml of blood was collected by vein puncture using Vacutainer tubes from each patient enrolled in this study for determination the level of IL-6 and IL-10 by using ELISA. The study showed that the highest mean level of IL-1 beta was found in women with breast cancer comparing with healthy control women (36.92±11.1 v.s. 10.1±3.7 pg/ml) (P: ≤0.05). The highest mean level of Il-6 was found in BC women comparing with control (97.13±14.7v.s. 22.13±6.91 pg/ml) (P: ≤0.05). The study showed that the highest mean level of IL-1 beta was found in women with breast cancer in the first stage of disease (33.2±9.1 pg/ml) and the level was still decreased to be the lowest level in the stage IV of breast cancer (20.18±2.9 pg/ml), the result was significant (P: ≤0.05). The study showed that the highest mean level of IL-6 was found in women with breast cancer in the first stage of disease (92.1±15.8 pg/ml) and the level was still decreased to be the lowest level in the stage IV of breast cancer (67.9±9.81 pg/ml), the result was significant (P: ≤0.05)

It was concluded that there was a highly significant relation of IL-1 beta and IL-6 with breast cancer and especially in first stage of disease

Keyword: *breast cancer; interleukin-1, interleukin-6*

Introduction

Breast cancer is the malignant tumor in which normal cells in the breast begin to grow without control and no longer die most commonly form the inner lining of milk ducts or the lobules that supply the ducts with milk [(1)]. In Iraq, breast cancer (BC) is the commonest type of female malignancy, accounting for approximately one third of the registered female cancers according to the latest Iraqi Cancer Registry [(2)]. As proposed by the World Health Organization (WHO), early detection and screening, especially when combined with adequate therapy, offer the most immediate hope for a reduction in breast cancer mortality [(3)].

Breast cancer is a heterogeneous disease witch results from a series of genetic and epigenetic events that lead to dysregulation of cell growth, circumvention of apoptosis, and development of the ability to invade the underlining tissue through the basement membrane[(4)]. The causes of these events remain largely unknown, although epidemiologic studies have implicated lifestyle, environmental, and germ-line genetic factors in predisposition to this disease. Familial forms comprise approximately 20% of all breast cancers and appear to have a distinctive pathogenesis dependent on the particular susceptibility gene involved[(5)]. Interleukin 6 (IL-6) is a proinflammatory cytokine, which is produced by a number of immune system cells; fibroblasts, macrophages, T and B Lymphocytes, endothelial cells, keratinocytes and tumor cells [(6)]. Interleukin 6 (IL-6), as major mediator of the inflammatory response, plays a primary role in the patho-physiology of cancer [(5)]. Cancer

cells exposed to IL-6 or which secrete the cytokine as an autocrine factor, show malignant features, such as an enhanced capacity to invade the extracellular matrix and an increased drug resistance [7]. Based on these data, the inhibition of the IL-6/IL-6 receptor interaction with specific antibodies has been proposed as a support cancer therapy [8,9]. The cytokines interleukin-6 (IL-6), tumor necrosis factor alpha (TNFalpha) and interleukin-1 beta (IL-1beta) are critical mediators of the inflammatory response. So the aim of the study was to estimate the level of IL-1 beta and IL-6 in women with breast cancer

Material and Method

A cross-sectional study was carried out in Kirkuk city from of November 2017 to March 2018. The number of breast cancer women under study were 55 women whose ages were between 30-70 years old. These patients admitted to Kirkuk oncology center. The control group who were matched to the breast cancer patients studied, included 35 unaffected women and their ages were between 30-75 years old. These women presented Kirkuk General Hospital Four ml of blood was collected by vein puncture using Vacutainer tubes from each patient enrolled in this study for determination the level of IL-6 and IL-1 beta by using ELISA technique.

Statistical Analysis

Computerized statistically analysis was performed using Mintab ver 18.0 statistic program. Comparison was carried out using Chi-square (X^2) for determination of the *P.* value.

Findings

The study showed that the highest mean level of IL-1 beta was found in women with breast cancer comparing with healthy control women (36.92±11.1 v.s. 10.1±3.7 pg/ml) (P: ≤0.05). The highest mean level of Il-6 was found in BC women comparing with control (97.13±14.7v.s. 22.13±6.91 pg/ml) (P: ≤0.05), Table 1.

Table 1: Level of IL-1 and IL-6 beta in breast cancer women and the control group.

Interleukins levels (Mean±SD) pg/ml	BC women	Control group	P. value
IL-1 beta	36.92±11.1	10.1±3.7	≤ 0.05
IL-6	97.13±14.7	22.13±6.91	≤ 0.05

The study showed that the highest mean level of IL-1 beta was found in women with breast cancer in the first stage of disease (33.2±9.1 pg/ml) and the level was still decreased to be the lowest level in the stage IV of breast cancer (20.18±2.9 pg/ml), the result was significant (P: ≤0.05). The study showed that the highest mean level of IL-6 was found in women with breast cancer in the first stage of disease (92.1±15.8 pg/ml) and the level was still decreased to be the lowest level in the stage IV of breast cancer (67.9±9.81 pg/ml), the result was significant (P: ≤0.05), Table 2.

Table 2: Comparison among Level of IL-1 and IL-6 beta levels regarding stage breast cancer

Interleukins levels (Mean±SD) pg/ml	Stage I	Stage II	Stage II	Stage IV	P. value
IL-1 beta	33.2±9.1	29.8±3.6	26.8±3.8	20.18±2.9	≤ 0.05
IL-6	92.1±15.8	80.6±13.2	77.8±10.8	67.9±9.81	≤ 0.05

Discussion

The role of other inflammatory cytokines (possibly TAM derived), IL- 1 was also addressed in breast carcinoma [10]. The role of the IL-1 system in human breast cancer is conflicting. Initial analyses regarding IL-1 indicated that its levels were significantly higher in invasive carcinoma than in ductal carcinoma in situ or in benign lesions, implying that elevated levels of IL-1 are directly correlated with a more advanced disease [6]. In addition, IL-1 has been shown to inhibit growth of breast cancer cells and to promote cellular differentiation in vitro, but it is equally known to stimulate the expression of several proteolytic enzymes in human cancer [11]. The consecutive degradation of extracellular matrix is a key element of local invasion and metastasis [12]. The mitogenic activity by IL-1 can be explained by induction of growthrelated oncogene (GRO) gene expression or induction of IL-8 expression via activation of the Nuclear factor B (NFκB) and activator protein (AP)-1 signal transduction pathways [13]. The robust response of the metastatic- or mesenchymal-appearing breast carcinoma cells to either IL-1 or TNF may be because of elevated expression of transcription factors needed for transcription of the IL-8 gene. TNF- B, a transcription factor, which can be activated by either IL-1, is an example of such a transactivator. Activated NF- B recognizes and binds to a consensus sequence in the promoter region of the IL-8 gene[14]. In this study, serum IL-6 level was evaluated in breast cancer patients as compared to healthy controls. Interleukin-6 is found to be elevated in various inflammatory and malignant diseases including metastatic breast cancer and their levels are found to correlate with the extent of the disease [9]. It is produced by some types of cancer cells and by normal stromal cells, such as fibroblasts and endothelial cells. By acting as growth factor, IL-6 is able to promote tumor cell proliferation through upregulation of anti-apoptotic and angiogenic proteins in tumor cells. Also IL-6, is a major mediator of the inflammatory response, plays a primary role in the pathophysiology of cancer [15]. The study results were in agreement with a recent study in Iraq made by Ayed [16] carried out on 75 breast cancer patients and 15 healthy controls. He found that serum IL-6 level for the patients was significantly higher than normal women and strongly correlated with disease progression. Also in Egypt, Ahmed *et al* [16] found that serum level of both IL-6 and IL-8 were found to be higher in patients than in healthy volunteers. In addition Sullivan [17] have mentioned that breast cancer patient serum and tumor IL-6 levels are clinically relevant, and therefore, should be routinely evaluated upon diagnosis . Statistically highly significant differences were seen in the Mean serum (IL-6) concentration of all disease stages as compared to the Mean (IL-6) serum concentration of the control group ($p < 0.01$). We suggest that higher mean serum (IL-6) level in early disease stage may be due to the high immune response of the body in this stage of the disease. It was reported that IL-6 antitumor activity was enhanced by induction of induction of T cell and B cell differentiation, stimulation of cytotoxic T cells and help in producing lymphokineactivated killer cells [18]. We thought that This immune response trigger synthesis and release of this cytokine leading to augmentation of its serum level that might be utilized as a marker of immunity status and immune system activation in prognosis and monitoring of the course of cancer [19]. This immune response might be attenuated with the progression of disease stage and cancer overwhelming which reflect decrease in (IL-6) synthesis. Our hypothesis could be supported by some literatures , mentioned that IL-6 and other IL-6-type cytokines are expressed in many primary breast tumors [20]. However, IL-6 expression is reduced in invasive breast carcinoma relative to normal mammary tissue and appears to be inversely associated with histological tumor grade [21]. The role of IL-6 in cancer progression is dependent on the balance of multiple pathways triggered simultaneously by the cytokine. However, concomitant stimulation of the cells by other endogenous or exogenous factors, even at low concentrations, may tip the balance toward one biological response, e.g., proliferation and antiapoptosis, or another, e.g., growth arrest and differentiation [22]. IL-6 has also been shown to influence the proliferation of normal and tumor-derived cells. IL-6 promote proliferation of hematopoietic progenitors, keratinocytes, myeloma/ plastocytoma, and Kaposi's sarcoma cells, whereas it inhibits the proliferation of M1 myeloid leukemia cells, early-stage melanoma cells, and lung and breast tumor cells. Thus, depending on the target cell, IL-6 induces various and sometimes contrasting biological responses[7-9].

Conclusions: There was a highly significant relation of IL-1 beta and IL-6 with breast cancer and especially in first stage of disease.

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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Production of Iodine-125 in Useful Medical Energy Possibilities

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Abstract

The goal of the present work is to achieve the nuclear data for medically important radioisotope Iodine-125. The Measured data of excitation functions for direct reactionsof particle induced nuclear reactions, which produced ^{125}I, are available in EXFOR library have been evaluated and recommended in this work. This evaluation is used to calculate the integral yield of radioactive Iodine-125. The average values was derived and used to calculate the most useful reaction for the production of Iodine-125which is ^{124}Te(d,n)^{125}Ireaction with no impurity, yield 0.611MBq (0.02mCi)/μAh, and useful energy range 5.5→7.5MeV from cyclotron using high enrichment for ^{124}Te.

Keywords: *enrichment, excitation functions, I-125, useful energy, yield*

Introduction

Iodine-125 is a medical radioisotope of Iodine. Its half-life is 59.49 days and it decays by electron capture(isomeric transition) with daughter ^{125}Te of spin 5/2+ (stable isotope). This state is not the metastable ^{125m}Te, but rather a lower energy state that decays immediately by gamma decay with a maximum energy of 35keV[1]. Iodine-125 used as nuclear imaging tracers to evaluate the anatomic and physiologic function [2]. Many studies have been developed for using Iodine-125 by Goolden et al. (1968) [3];Venikov et al. (1993)[4]; Bastian et al. (2001) [5]; Hohn et al. (2001) [6]; Audi et al. (2003) [7], Weinreich and Knust (2005) [8]; Gul (2009) [9];Uddin et al. (2011) [10]; Augustine et al. (2013) [11]; Al-Alawy and Mohammed (2016) [12]and Solovev and Merkusheva (2017) [13] for different applications in diagnostic and treatments by studying the excitation functions, enrichments, differential and integral yields, useful energy and impurities levels.Iodine-125 is commonly used by radiation oncologists in low dose rate brachytherapy in the treatment of cancer at sites other than the thyroid (using positron emission tomography (PET)), especially in prostate cancer[14, 15].

Theoretical Basics

In the scope of this work, the stopping powers are programmed and calculated using the Ziegler formulae expressions valid for the energy range as follows [16, 17, and 18]:

1- Energy range (1-10)×10^{-3} MeV $-\frac{dE}{dx} = A_1 E^{1/2}$ (1)

2- Energy range (10-999) ×10^{-3} MeV

$$\left(-\frac{dE}{dx}\right)^{-1} = \left(-\frac{dE}{dx}\right)_{Low}^{-1} + \left(-\frac{dE}{dx}\right)_{High}^{-1} \quad (2)$$

$$\left(-\frac{dE}{dx}\right)_{Low} = A_2 E^{0.45} \text{ and } \left(-\frac{dE}{dx}\right)_{High} = \left(\frac{A_3}{E}\right)\ln\left[1+\left(\frac{A_4}{E}\right)+A_5 E\right] \quad (3)$$

3- Energy range (1000-100.000)×10^{-3} MeV

$$\left(-\frac{dE}{dx}\right) = \left(\frac{A_6}{\beta^2}\right)\left[\ln\left(\frac{A_7\beta^2}{1-\beta^2}\right) - \beta^2 - \sum_{i=0}^{4} A_{i+8}(\ln E)^i\right] \quad (4)$$

Where: E is the proton or deuteron energy in (MeV), A_i are the coefficients given by Ziegler, β is the ratio of incident corpuscle velocity and the velocity of light. Hence, the stopping powers are also programmed and calculated using the Ziegler formulae expressions valid for different energy range of incident alpha as follows[16, 17, and 18]:

1- Energy range 1kev – 10MeV $\left(-\frac{dE}{dx}\right)^{-1} = \left(-\frac{dE}{dx}\right)_{Low}^{-1} + \left(-\frac{dE}{dx}\right)_{High}^{-1}$ (5)

$$\left(-\frac{dE}{dx}\right)_{Low} = A_1 E^{A2} \text{ and } \left(-\frac{dE}{dx}\right)_{High} = \left(\frac{A_3}{E/1000}\right)\ln\left[1+\left(\frac{A_4}{E/1000}\right)+\left(\frac{A_5 E}{1000}\right)\right] \quad (6)$$

2- Energy range >10 MeV

$$\left(-\frac{dE}{dx}\right) = \left(A_6 + A_7 EE + A_8 EE^2 + A_9 EE^3\right) \quad (7)$$

Where $EE = \ln(1/E)$ and A_i (i =1 – 9) are the coefficients given by Ziegler [16, 18]: for incident alpha.For any energy E, the Yield of product nuclei (activity in Bq) can be expressed as the function of the cross section as[19]:

$$Yield = I(\phi\ n)\ H\ \left(1-e^{-\lambda t}\right)\int_{E_{out}}^{E_{in}} \sigma(E)\left(\frac{dE}{dx}\right)^{-1} \quad (8)$$

Where I is current of projectile in (μA), *ø*is beam flux in (s^{-1}), n is number of atoms per unit volume, N is Avogadro's number, A is the mass number of the target in (amu), H is isotopic abundance (or enrichment) of the target, λ is decay constant of the product in (h^{-1}), t is time of irradiation in (h), and $\sigma(E)$ is cross section at energy E in (mb).

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Data Reduction and Analysis

Table 1 shows the agreement of recommended energy range with the international EXFOR library energy range used in the present work for available measuring data for proton, deuteron and alpha particle induced reactions for the ^{125}I production. Table 2 shows the results of the calculated threshold energies and Q-values compared with the experimental values which are taken from National Nuclear Data Center (NNDC) [20]; both are in a very good agreement. The table also provides a natural and enriching abundance of the target material.

Table 1: Energy range recommended for iodine-125 production

Target Element	Reaction	Product	Energy Range (MeV)	Author's Ref. No.
^{125}Te	(p,n)	^{125}I	5.5−100.5 5.5−100.5	Hohn et al. (2001) [6] (pw)
^{124}Te	(d,n)		5.8−14.1 5.8−14.1	Bastian et al. (2001) [5] (pw)
^{123}Sb	(α,2n)		14.8−39.6 14.8−39.6	Uddin et al. (2011) [10] (pw)

Table 2: Nuclear properties of the reactions that produce iodine-125

Reaction Process used	Threshold Energy (MeV) [20]	Q-Value (MeV) [20]	Target Material		
			Isotope	Natural Abundance% [21]	Enrichment %
^{125}Te(p,n)	0.975926(0.0605) 1.487301(pw)	-0.968117(0.06) -1.475403 (pw)	^{125}Te	7.07	98.3[6]
^{124}Te(d,n)	0.0(0.0) 0.0(pw)	3.3762842(0.07) 2.874795 (pw)	^{124}Te	4.74	99.8[5]
^{123}Sb(α,2n)	14.34 15.608386 (pw)	-14.11 -15.116233 (pw)	^{123}Sb	42.7	98.28[10]

Method Used to Obtain the Recommended Cross Section

The interpolations of the nearest data for each energy interval as a function of the sets of experimental cross sections and their corresponding errors for each value (with different energy intervals) are re-arranged according to the energy interval 0.01MeV for each author. The normalization for the statistical distribution of cross sections errors to the corresponding cross section values for each author has been done. The interpolated values are calculated to obtain the recommended cross section which is based on the weighted average method[22]:

Findings and Discussion

Recommended Cross Sections

The experimental results in the International Atomic Energy Agency (IAEA); (EXFOR) library leaves little doubts that the hypothesis of cross section gives an excellent account of many diverse types of nuclear reactions. The features of iodine production nuclear reactions induced by protons, deuteron and alpha mainly on the $_{52}$Te and $_{51}$Sb target elements have been evaluated. It is important to note that the energy range of the reaction, taken from different authors, is not identical.

For this reason, the determination of the energy range has been processed. The results for each reaction are discussed as follows:

^{125}Te(p,n)^{125}I Reaction

The measured data for the cross sections of this reaction reported by Hohn et al. (2001) [6], interpolated, and recalculated in fine steps of 0.01MeV from 5.5MeV up to 100.5MeV for incident proton.

^{124}Te(d,n)^{125}I Reaction

The measured data for the cross sections of this reaction reported by Bastian et al. (2001) [5], interpolated, and recalculated in fine steps of 0.01MeV from 5.8MeV up to 14.1MeV for incident deuteron.

^{123}Sb(α,2n)^{125}I Reaction

The measured data for the cross section of this reaction reported by Uddinet al. (2011) [10], interpolated, and recalculated in fine steps of 0.01MeV from 14.8MeV up to 39.6MeV for incident alpha.

Our trails were directed to fit the evaluated cross sections for the data taken from EXFOR library. Using the recommended cross sections as an input data, a Matlab-8 has been executed to obtain a Polynomial fitting expression and the fitting parameter of the fit formula with lower chi squared value (χ^2_{min}).

Stopping Power and Calculated Yield

The stopping power of target elements for proton, deuteron and alpha particles has been calculated in the present work using SRIM (2003) [23], as an experimental results and Ziegler equations (1→7) as a theoretical calculation results. For (p,n), (d,n) and (α,2n) reactions calculations. Therefore, the calculated yield for ^{125}I has been calculated using equation (8). The main aim of this study is to increase calculated yield from these reactions by increasing the energy of proton, deuteron or alpha beams which can interact with different targets.

Useful Energy and the Production Yield

The excitation function for the production of iodine radioisotopes in natural Tellurium target for 10 to 35MeV of incident proton is measured byOropeza et al. (1994) [24] andArcerbitet al. (1974) [25]. Table 3 shows the cyclotron production of ^{125}I and the reaction contribution as a function of the energy range of incident particles on the Antimony ^{123}Sb and Tellurium (^{124}Te, ^{125}Te) targets elements. After certain degree target enrichment, the following sequences are to be performed: In radioisotope production from a nuclear reaction, the optimization of production routes is needed. This process involves a selection of the projectile energy range that will maximize the yield of the product and minimize that of the radioactive impurities. With particular emphasis on the data needed for the production of medicalsignificance radionuclide ^{125}I.

Table 3: Useful energy and the production yield for the production of iodine–125

Target Element	Reaction	Useful Energy (MeV)	Yield MBq(mCi)/μAh	Radioiodine impurity %	Author's Ref. No
^{123}Sb	(α,2n)	20→30 20→30	——— 1.64(0.044)	——— (0.059)^{126}I, (2.411)^{124}I, (0,23)^{126}I	[10] (PW)
^{124}Te	(d,n)	5.8→7.5	0.613 (0.017)	No Impurity	(PW)
^{125}Te	(p,n)	7→18 5.5→10	——— 2.63 (0.071)	——— No Impurity	[3] (PW)

Incident proton on enriched ^{125}Te target to produce ^{125}I

The production of $^{123,124,125}I$ has been carried out byHohn et al. (2001) [6]. Hohnet al. performed that the^{125}Te target was an isotopic enrichment with different compositions as; ^{120}Te(0.1%); ^{122}Te(<0.1%); ^{123}Te(0.5%); ^{124}Te(0.8%); ^{125}Te(98.3%); ^{126}Te(0.1%); and ^{130}Te(<0.1%). The recommended cross section of the^{125}Te (p,3n)^{123}I, ^{125}Te(p,2n)^{124}I, and ^{125}Te(p,n)^{125}I reactions over the entire energy range 21.5−68.2MeV, 10.6−100.5MeV, and the 5.5−100.5MeV respectively were calculated. The excitation functions are plotted in Figure1.

The useful energy range for the production of ^{124}I is carried out over the interval of E_P=15 to 20MeV, which is agree with the result of Koehler et al.(2010) [26]. The calculated yield should be 91.13*MBq* (2.46*mCi*)/*μAh*, which is also agree with Ref.[26]. Theproductionof ^{125}Iwithin this energy range is regarded to be as an impurity of 21% with ^{124}I.

The useful energy range for the production of ^{125}I is carried out over the interval of E_P=5.5 to 10MeV, with no impurity, but with very low calculated yield 3.35MBq (0.091mCi)/μAh. While the useful energy ranges for the production of ^{123}I may be carried out over the interval of E_P=30 to 40MeV, with an impurity of 44% of ^{124}I and 1.5% of ^{125}I. The calculated yield is 297.401MBq (8.04mCi)/μAh, for ^{123}I. This high impurity and high energy need high cost for cyclotrons.

Incident deuteron on enriched ^{124}Te target to produce ^{125}I

The production of ^{123}I ,^{124}I , and ^{125}I have been carried out by Firouzbakht et al. (1993) [27] and Bastian et al. (2001) [5]. The recommended cross section of ^{124}Te(d,n)^{125}I, ^{124}Te(d,2n)^{124}I, and ^{124}Te(d,3n)^{123}I reactions over the entire energy range 5.8−14MeV, 7.5−23.6MeV, and 7.5−23.6MeV respectively were calculated. The excitation functions are plotted in Figure2.

Bastian et al. performed that the^{124}Te target was an isotopic enrichment with different compositions as:^{123}Te (0.2%) and ^{124}Te (99.8%) for ^{124}Te(d,2n)^{124}I, and ^{124}Te(d,3n)^{123}I reactions. While Firouzbakht et al. gave no data for the enrichment. This useful energy range for the production of ^{123}I is Ed=18 to 22.5MeV. The calculated yield should be 45.9MBq (1.2mCi)/μAh. The production of ^{124}I in this energy range is regarded to be an impurity of 26% with ^{123}I. It can be seen from Figure2, that below 18MeV the contamination of ^{124}I is 100% while the energy range Ed=5.5 to 7.5MeV can be regarded as useful energy for the production of ^{125}I without any impurities.

Incident Alpha on enriched ^{123}Sb target to produce ^{125}I

The production of ^{123}I has been carried out by Ismail (1990) [28], Singh et al. (1991) [29], and Al-Alawy and Mohammed (2016) [12], while the production of ^{124}I has been carried out by Watson et al. (1973) [30], Ismail (1990) [28], Singh et al. (1991) [29], and Uddin et al. (2011) [10]. ^{125}I has been carried out by Uddin et al. (2011) [10], and Al-Alawy and Mohammed (2016) [31]. While the production of ^{126}I (half-life=13d) has been carried out by Ismail (1990) [28], Singh et al. (1991) [29], Singh et al. (2006) [32] and Uddin et al. (2011) [10]. The recommended cross section of ^{123}Sb(α,4n)^{123}I, ^{123}Sb(α,3n)^{124}I, ^{123}Sb(α,2n)^{125}I, ^{123}Sb(α,n)^{126}I reactions over the energy range 34.4−58.4MeV, 9.72−58.8MeV, 14.8−39.6MeV, and 9.7−40.2MeV were calculated. The excitation functions are plotted in Figure3.

Singh et al. used enriched (99.9%) for ^{123}Sb target, whileIsmail gave no data for enrichment. Watson et al. used enriched (99.999%), andUddin et al. used enriched (98.28%) of the target. It can be seen that the useful energy range for the production of ^{124}I is carried out over the interval of Eα=32 to 40MeV. The calculated yield should be 11.4*MBq* (0.31*mCi*)/*μAh*, which can be compared with the result obtained by Uddinet al. of 11.7*MBq* (0.32*cmCi*)/*μAh*. Therefore, below 32MeV, the production of ^{125}I and ^{126}I are regarded to be an impurity of 23% and 13.2%, respectively with ^{124}I. Above 40MeV, the production of ^{123}I is regarded to be an impurity of 101.2% with^{124}I. Hence, the useful energy ranges for the production of $^{124}I$is selected to be Eα=32 to 40MeV, with a minimum impurity 4.8%, 2% and0.2% of ^{123}I, ^{125}I, and ^{126}I respectively.

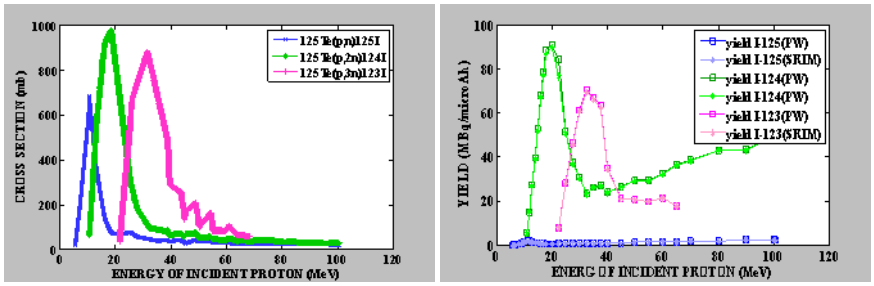

Figure1 Left side: Recommended cross sections of (p,n), (p,2n), and (p,3n) reactions on ^{125}Te, versus incident proton energy. Right side: Calculated yield of the incident proton in ^{125}Te.

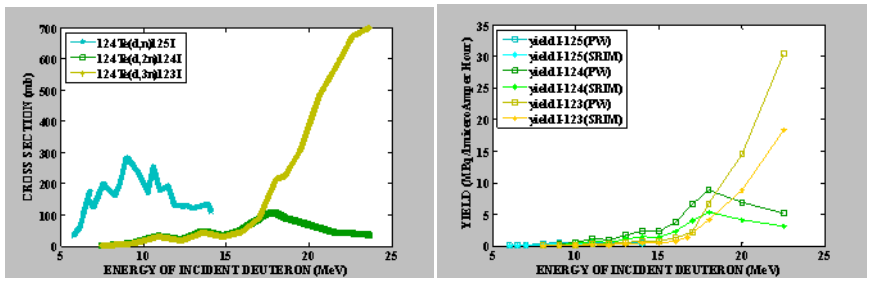

Figure2 Left side: Recommended cross sections of (d,n), (d,2n), and (d,3n) reactions on ^{124}Te, versus incident deuteron energy. Right side: Calculated yield of the incident deuteron in ^{124}Te.

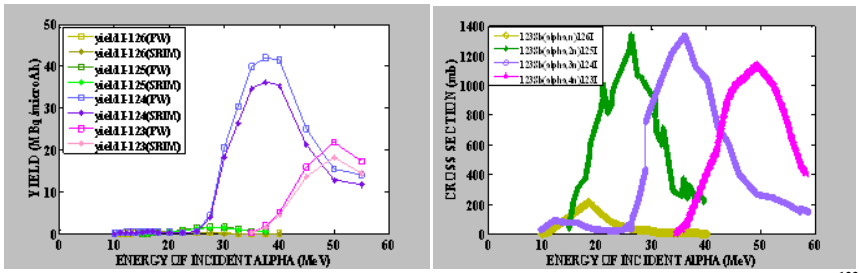

Figure3 Left side: Recommended cross sections of (α,4n), (α,3n), (α,2n), and (α,3n) reactions on ^{123}Sb, versus incident alpha energy. Right side: Calculated yield of the incident alpha in ^{123}Sb.

Conclusion

Although higher enrichment of the targets ($_{51}$Sb, $_{52}$Te) has been used, for the production of Iodine isotopes, to reduce the appearance of impurities, this impurity not disappears. So that, the area of the energy range for the cyclotron must be specify to obtain a higher production of radioactive Iodine required with less percentage of impurity.

The characteristic of the diagnosis and treatment radioisotopes is to ensure the access of radiation to the organ for diagnostic or treated without moving to the tissue. Therefore, among three reactions for the production of ^{125}I (^{125}Te (p,n), ^{124}Te(d,n), ^{123}Sb(α,2n)); the most useful reactions are:

For incident deuteron: The ^{124}Te(d,n)^{125}I reaction with no impurity, with yield 0.611MBq (0.02mCi)/μAh and the useful energy range 5.5→7.5MeV.

For incident proton: The ^{125}Te(p,n)^{125}I reaction with no impurity, with yield 3.35MBq (0.091mCi)/μAh and the useful energy range 5.5→10MeV.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Physics, College of Science, Mustansiriyah University, Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

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Detection of Mycoplasma Pneumonia Infection in Sinusitis's Patient

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Abstract

"This study aims to detecting the probability of M. pneumonia prevalence in sinusitis patients the study in rolled (178) patients had a history of respiratory infection.Were included (135) patients suffering from sinusitis disease and (43)Control group of patients had no radiological evidence of sinusitis disease.The range age in cases was (18-62) years with a mean of(40) years , in controls it was 18-51 years witha mean of(34.5).

Blood samples were examined for M. pneumonia antibodies IgG and IgM by enzyme linked immune sorbent assay (ELISA). Elevated antibody titers were found in (124) patients (80%),and in 31control (20%). Acute and previous immunities "M. pneumonia IgM and IgG antibodies " were observed in (17%) and (67.7%) of cases, and (2.5%) and (12.2%) of controls, respectively.

Finding demonstrate that the predominant M. pneumonia antibodies type "IgG, IgM" in sinusitis patients and symptoms like headache and fever. The study showed that the positivity rate of M.pneumoniae infection reach peak level in crowding index (+4) and (3- 3.9) in both case and control.

Keywords : *M. pneumonia, antibodies , IgG, IgM, enzyme linked immune sorbent assay, sinusitis patients, crowding index.*

Introduction

"Mycoplasma pneumonia living bacteria which can cause upper respiratory tract infections, including pharyngitis, sinusitis, ear pain are extremely small self-replicating free-rhinorrhea and pneumonia[1][2]. *M. pneumonia*e is described as atypical and accounts for 1–29% of community-acquired pneumonia cases"[2]. "The causative bacteria can be transmitted through aerosols as well as in settings which promote close physical contact, such as homes, schools, military barracks and dormitories"[3]. "The post-exposure incubation period is between two to three weeks and infections are more prevalent among children and young adults"[4]. "Common risk factors include age (i.e. younger children or older adults), immune status (i.e. immuncompromised individuals with HIV or those undergoing chemotherapy or taking steroids), smoking and pre-existing lung disease"[5]."Sinusitis, also known as rhinosinusitis, is inflammation of the Paranasal sinuses. It can be due to infection, allergy, or autoimmune problem. Most cases are due to a viral infection and resolve over a course of 10 days. It is a common condition, with over 24 million cases in the unites states"[6]."Evidence of maxillary sinusitis has been found in human archeological specimens discovered in Africa, north America, and Europe"[7]."Reported association between the M. pneumonia and sinusitis a range of various disorder similar in nasal and Paranasal sinuses mucosa inflammation for as long at least 12 consecutive weeks are called chronic rhinosinusitis. *M. pneumonia* was previously detected in human nasal which implicates *M. pneumonia* as a causative agent in the etiology of sinusitis"[8].

"Because of our concern about the possibility of unrecognized *M. pneumonia* infection among different group of population and to determine the etiology of *M. pneumonia* among these groups the study aimed as following:

Prevalence study of *M. pneumonia* IgM and IgG antibodies by ELISA test.

A comparative study for the occurrence of *M. pneumonia* infection in different population groups."

Material and Method

Patients, controls, place, and time

"The study design consist of(178) patients had a history of respiratory infection with onset of symptoms (1 to 20) days before inclusion in the present study. (135) patients suffering from sinusitis disease attending ENT clinic and Baghdad hospital. Control group of (43) patients were visited an ENT clinic with comparable age and had not radiological evidence of sinusitis disease . All samples from patients and controls were collected from beginning of December 2017 to the end of July2018.**"**

Specimens :

Collection of serum sample:

"3-5ml of blood sample was collected, centrifuge for ten minutes x 3000 rpm. Serum dispensed in aliquots' of 0.5 ml. all sera were immediately frozen at -20c until used. This was used for detection of IgG and IgM antibodies to Mycoplasma pneumonia in human serum. The evaluation of specific M. pneumoniaIgG and IgM antibodies were carried out with commercial kits (Chemi-Con- Germany)."

Statistical Analysis:

"Quantitative variable were described as frequencies and percentage for each group. Statistical analysis including, Chi square values and p values were calculated for all categorical variables. "

Finding

Table(1) Seroprevalence of Mycoplasma pneumonia among study group

Study group	M.pneumonia IgG no.(%)	M.pneumonia IgM no.(%)	Total No.(%)
Patient	105(67.7)	19(12.2)	124(80)
Control	27(17)	4(2.5)	31(20)
Total	132(84.7)	23(14.7)	155(100)

Table (1)demonstrate that Acute infection IgM antibodies was detected in (12.2%) of cases and (2.5%) of controls. And for previous immunity IgG antibodies were (67.7%) and (17%), respectively

Table(2) clinical symptoms associated with Mycoplasma pneumonia

Symptoms	Patient		Control	
	M.pneumoniae IgG antibodies No(%)	M.pneumoniae IgM antibodies No(%)	M.pneumoniae IgG antibodies No(%)	M.pneumoniae IgM antibodies No(%)
Headache	56 (45)	25 (20)	6 (19.3)	3 (9.6)
Fever	43 (34.6)	11 (8.8)	18 (58)	8 (25.8)
Throat pain	17 (13.7)	6 (4.8)	4 (12.9)	-
Fatigue	32 (25.8)	12 (9.6)	7 (22.5)	2 (6.4)
Dry cough	88 (70.9)	31 (25)	-	-
sputum	12 (9.6)	2 (1.6)	9 (29)	4 (12.9)

Table (2) show the most common symptoms in patients with this atypical pathogen " M.pneumoniae" were headache

and dry cough in both IgG and IgM antibodies. While in control group the most common symptoms were fever and sputum.

Table(3) Distribution study group according to the crowding index

Crowding index	Patient		Control		total
	Positive M.pnumonia antibodies	Negative M.pnumonia antibodies	Positive M.pnumonia antibodies	Negative M.pnumonia antibodies	
1-1.9	21(16.9)	5 (45.5)	5(16.2)	6(50)	37(20.7)
2-2.9	16 (12.9)	4 (36.3)	3(9.6)	4(33.3)	27(15.2)
3-3.9	42 (33.9)	-	12(38.7)	2(16.7)	56(31.5
4+	45 (36.3)	2 (18.2)	11(35.5)	-	58(32.6)
Total	124(100)	11(100)	31(100)	12(100)	178(100)

As shown in table (3) that the high M. pneumoniae infection was seen insinusitis patients and control living with crowded family(3-3.9 and +4) .

Table (4) Respondent study group about practices regarding mycoplasma infection

practices	Patient		control	
	Yes No.(%)	No No.(%)	Yes No.(%)	No No.(%)
Stay away from people who have symptoms of mycoplasma	29(21.4)	106(78.6)	33(76.7)	10(23.3)
Wash hand before eating	113(83.7)	22(16.3)	40(93.1)	3(6.9)
Cover the nose and mouth when they sneeze or cough	17(12.6)	118(87.4)	29(67.4)	14(32.6)
Eat a balanced diet	98(72.6)	37(27.4)	32(74.4)	11(25.6)
Get 6-8 hrs. Of sleep night	54(40)	81(60)	15(34.8)	28(65.2)

With respect to some practices regarding Mycoplasma pneumoniae infection table (4) show that the Most of the study patients (87.4%) were answered no cover the nose and mouth when they sneeze or cough followed by 106(78.6%) the answered no stay away from people who have symptoms of mycoplasma pneumoniae infection.

Discussion

(80%) of the patients showed a fourfold or greater rise of antibodies to Mycoplasma pneumonia (table 1). In the control group, a corresponding rise in antibody titer was seen in 20%, acute infection (IgM)was detected in (12.2%) of cases and (2.5%) of controls. These number for previous immunity (IgG) were (67.7%) and (17%), respectively. Antibodies in serum (IgM, IgG) had shown significant difference (p>0.05) between case and control).

"Current study shows that M.pnumonia has a possible etiologic role in development of Sinusitis patients, Our result acceptable agreement with other

study that suggest the respiratory M. pneumonia may be potential etiological agent in a sinusitis alone or in combination with the common bacterial pathogens of sinusitis"[9].

"Other study finding that no significant difference between case and control"[10]." The incidence rate of mycoplasma antibody in our study is lower than that of other study that detected M. pneumonia in (93%) of sinusitis patients but was seen only in (14%) of the control group this may be due to we use serum instead of DNA- PCR for diagnosis of active M.pnumonia"[11]. "M. pneumonia is known to produce a gradual tracheobronchitis with malaise and non-productive cough, which can progress to pneumonia and extra pulmonary manifestations"[12][13].

"In our study dry cough and headache were the most common symptoms observed for patients with samples positive for M. pneumonia antibody IgM, IgG. However the fever and sputum the most predominant symptom found in control group. our study came in a agreements with other study that found that both cough and fever were the most common symptoms"[14].

"In this study, overcrowding were associated with a history of M. pneumonia infection (table 3), our study finding patients from household with 1 or 2 people per room were less likely to have M. pneumonia infection compared to patients from households with more than 3 people per room. other study found consistent evidence of an association between bed sharing with someone with cough in sever and non-sever pneumoniae"[15].

"This due to M. pneumonia is spread when a person who is sick cough or sneezes while in close contact with others who then breath in the bacteria. Most people who are exposed for a short amount of time do not get sick. However ,it›s common for this illness to spread between family members wholiving together"[1]." Regarding practice of mycoplasma infections, the present study revealed most of studygroup was noStay away from people who have symptoms of mycoplasmaAnd no Cover the nose and mouth when they sneeze or cough,the causative agent can be transmitted through aerosols as well as in setting which promote close physical contact, such as homes, schools, military barracks and dormitories"[2].

Conclusion

"M. pneumonia might be of importance in the etiology of sinusitis patients. Macrolides potential to reduce the virulence of some bacteria may be an important feature in reducing tissue damage in cases with chronic infection.ىc"

Conflict of Interest: Non

Source of Funding : Self

Ethical Clearance: Were taken Approval From the specialist doctor and patients.

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Immunopathological Study of *Brucellaabortus* in White Mice Immunize with Killed Antigen

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Abstract

The study was aimed to evaluate the protective efficiency of the formalin killed *Brucellaabortus*antigen against *Brucella* infection in mice . Inorder to achieve of thisgoal.Fifty white mice were divided into three groups randomly. The 1st group (20 mice) was immunized 0.3ml of formalin killed *Brucellaabortus*antigen, two doses, two weeks interval, the 2ndgroup (20 mice) was served as control positive while 3rd group (10 mice) was inoculated with 0.3ml of sterile normal saline and it served as control negative group.At 30 day post immunization, the 1st and 2nd group were inoculated I/P with 0.3ml of bacterial solution containing $1X10^8$cfu/ml, I/P all animals were sacrificed at day 30 post infection,blood samples were collected for measurement IL 4,1L six and TNFa, and small pecies from internal organs were fixed in I0 percentage of formaline for histopathologicalexamination.The results showed that the serum levels of IL-4, IL-6and TNFα were (36.22±3.81),(29.74±2.79) and (161.214±2.98) respectevlly. The results of histopathological changes showed severe pathological changes including granulomatous lesions in the liver parenchyma and dilated of sinusoids with fibrin networks deposition, neutrophils infiltration in the lung and RBCs in thickness pleura,the spleen showed depletion of white pulp and congested red pulp as well as shownacute cellular degeneration in epithelial cells of kidney, atrophy of glomerula tufts, with dilated of Bomana spaces and hemorrhage in capsular region,while immunized infected animalswere showed mild to moderate cytopathological effect with mononuclear cells aggregation around blood vessels of examined organs particularly liver. we concluded that killed Brucellaabortous antigen can stimulated good cell mediated immune response against *Brucellaabortous* infection of mice.

Key Words:*Brucellaabortus , Immunopathological , Antigen ,Histopathological, Interleukin.*

Introduction

The epidemelogy of zoonotic disease brucellosis that caused by *Brucella*about(500,000) people in year[1]. Majar causal agent is*Brucellamelitensis*, *Brucellasuis*, and *Brucellaabortus*. There are different mode of infection method like inhalation of infected aerosols. Many studies concluded that airborne transmission consider major method of infected human with diseasethat result from bovine and porcine slaughterhouses, preparation of vaccines as well as rural places[2–6].*B. abortus*isis a pathogenic bacteriacan live in the intracellularenvironment. Inside cells, there exist adverse conditionsincluding acidic pH, proteases, and reactive oxygen species(ROS) and reactive nitrogen species (RNS) [7].This bacterium contain lipopolysaccharide (LPS) in cell wall structure that play important role in pathogenicity [8]. Lipid A protein contain large amount of fatty acidmoleculesthat act on decrese immune response of the human body through serve as a poor TLR4 agonist. O-polysaccharide portion that consider cytotoxic of macrophage is absence in content of lypopolysaccaride.The long chain of fatty acid in lipid A neded more experimntale evidences. Reduce activation of TLR4 led to enhanced uptake observedwith rough Brucellathrough enhanced activity of PI3K[9].

Vaccine play important role in activated two arms of immune response that play crucial role in eradication

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of Brucella infection, live attenuated vaccine elicited efficient protective immunity against Brucellaabortus but these vaccine may associated with persistent infection or reversion their virulence[10] ,therefore application other type of vaccine antigens are safety when used in the human immunization these vaccine include killed Brucella antigens ,crude extracts , subunit and DNA vaccine[11].Therefore the aim of the current study was to determine the efficiency of killed Brucellaabortousantigen in the stimulatedprotecive immune response against infection by virulent *Brucellaabortous* strain.

Materials and Method

1- Bacterial isolates

Brucellaabortus was obtained from advanced biotechnology lab. and diagnosed by biochemical tests according to [12].

2- Cultural media

Thecultural media were used this study brucella agar, blood agar,Tryptic soya agar. The media were prepared according to instruction of the producer company.**3-Antigens(killed Brucellaabortus antigen)**

Prepared by Hiallibartion and Blazkovec (1975)[13] as follows: The activated and purified bacteria was planted on the heart and brain broth and incubated at 37 ° C for 24 hours. Examine bacterial growth to ensure purity by preparing a glass slide and chromed dye.Transfer 4 ml of the broth and plant on the center of the acre and cut the heart and brain and distribute this quantity equally on the surface of the plant center by moving the dishes and then left to dry after the dishes were transferred to the incubator and left 48 hours. Take the non-contaminated dishes and the bacteria were harvested as follows:Harvested with PBS solution and placed in test tubes.The total number of bacterial harvesting was calculated in Colonies Forming UintCFU / ml[14].After washing the microbial growth by centrifugation at 3000 cycles / min for 30 minutes using PBS and two consecutive times and then discard the floating fluid.The neutralized formalin solution was added to the harvest to the original volume level and the implant was left for 24 hours to ensure homicide. The concentration was for the formalin solution (0.03% - 0.05%).Wash bacterial growth twice by PBS solution and using centrifuge at 3000 rpm for 15 minutes.Ensure that killing is done by taking part of the growth by means of a vector of germs and laying on the blood acar medium and it is ascertained that there is no bacterial growth.The precipitate was suspended using PBS solution and stored in sterile bottles until use.

4- Challenge dose

The *Brucellaabortus* was cultured ,growth and purification on the media of this study , the live bacterial cell counted according to [14].

5- Cytokine assessment

Interleukin-4, Interleukin-6 and TNFα Assay Procedure, The procedure is performed at room temperature according to manufacturer's instructions (Boster´s –Korea).

6- Experimental design

Fifty white mice were used (male and female), the ages (8-10weeks) and their weight ranged between (25-30gram), obtained from institute of sera and vaccines \ ministry of health, and reared in cages furnished clean sawdust , and fed concentrate feed during the duration of the experiment. The white mice were divided into three groups were first group includes 20 mice immunized inoculated with 0,3ml of formalin killed antigen,,two dose ,2weeks intervals at day 30 post immunization, and it was inoculated I/P with $1X10^8$cfu /ML.The second group includes 20 mice was considered as positive control.it was inoculated I/P with as 1stgroup. Third group includes 10 mice was inoculated I/P with 0.3ml ml of sterile normal saline ndservedas control negative group.At day 30 post infection ,all animals were sacrificed and blood samples were collected for determine cell mediated immune response by measurement of cytokines including TNF a,IL 4 and IL 6 and small pieces were fixed in 10 formalin for Histopathological examination according to [15].

Funding

Immune response

The cellular immune response

The results were revealed that serum levels of IL-4,IL-6 and TNF-α were 36.22±3.81, 29.74±2.79 and 161.214±2.98respectively as shown in table 1.

Table 1: Cytokine profile of*Brucellaabortus* in immunized mice post infection

groups	IL-4(pg/ml) mean±SE	IL-6(pg/ml) mean±SE	TNF-α (pg/ml) mean±SE
G1immunized group	36.22±3.81	29.74±2.79	161.214±2.98
G2 positive group	28.12±2.65	20.61±1.56	113.325±3.172
G3 negative group	4±0.23	2.5±0.31	21.4±0.578

Histopathological examination:

Non-immunized infected animals at day 30 post-infection

Section in the liver of animal at 30 days post infection showed of kupffer cells proliferation , with granulomatous lesions consisting from aggregation of active macrophages and lymphocytes in the liver parenchyma and dilated of sinusoids (Fig.1).

Lung Anatomy

After 30 days of infection, the anatomy of lung showed revealed fibrin networks deposition, neutrophils. infiltration and RBCs in thickness pleura (Fig.2).

Spleen Anatomy

After 30 days of infection, the anatomy of spleen showed depletion of white pulp and congested red pulp with inflammatory cells infiltration (Fig.3).

Kidney Anatomy

In antomy of kidney was clearly acute cellular degeneration of epithelial cells and shownatrophy of glomerula tufts, with dilated of Bomana spaces with hemorrhage in capsular region(Fig.4).

Immunized infected animals

In immunized infected animal, The pathological effect was were mild to moderate with mononuclear cells aggregation around blood vessels of examined organs particularly liver.

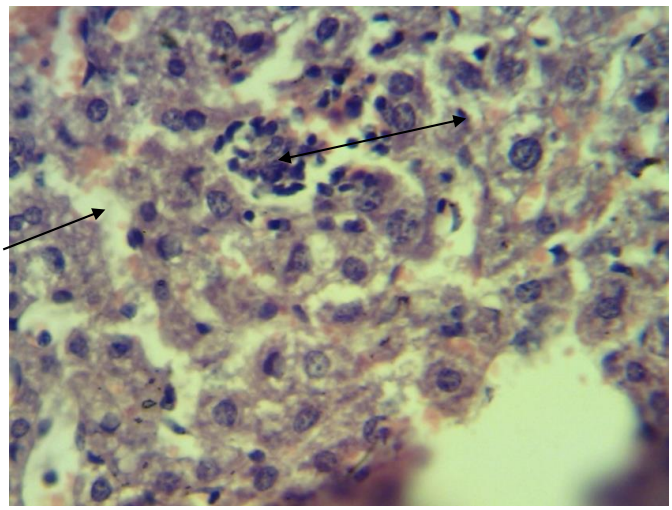

Figure 1. Anatomy of animal liver after infection that explanedproliferation of kupffer cells and granuloma that result from accumulation of active macrophages and lymphocytes in the liver parenchyma and dilated of sinusoids (H & E stain 400X).

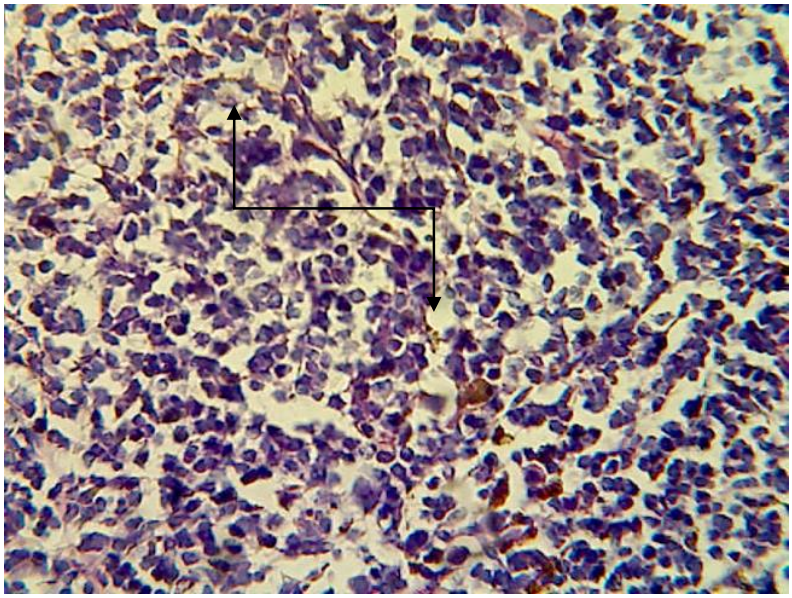

Figure3.Anatomy of animal spleen after infection showed depletion of white pulp and congested red pulp with inflammatory cells infiltration (H & E stain 400X)

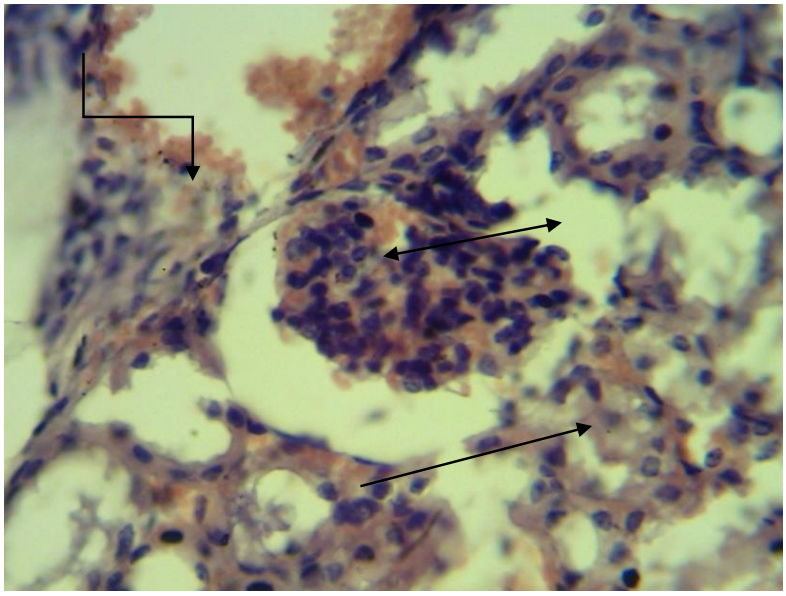

Figure4.Anatomy of animal kidneyafter infection interpreted of acute cellular degeneration in epithelial cells, atrophy of glomerular tufts, with dilated of Boman spaces with hemorrhage in capsular region (H & E stain 400X)

Discussion

Brucellaabortus is intracellular zoonotic pathogen cause brucellosis in human and wide range of animals [16] ,the pathogenesis of Brucella was dependent on its ability intracellular survival and replication in the vacuolar phagocytic compartments of macrophages [17], therefore cell mediated immunity play crucial role in eradicated the Brucellainfection. The current study revealed high levels of serum TNFa in immunized animals as comparedwith those levels in non immunized animals post infection,these result may indicated that the killed Brucella antigens may stimulated better cell mediated immune response against Brucellaabortous infection, these idea was agreent with [18] who demonstrated that the TNFa play important role in host defense mechanisms through activated microbicidalactivity of macrophages and neutrophils in addition it act on NK cells with IL 12 to produced INF y.protective immunity against Brucella can mediated by cytokines, INF y which produced by active Th1 cells that mediated by activated NK cells to produce INF y, these cytokine can facilitate differentiated of Tho cells to Th1 that additionally produce INFy, activator macrophages , also TNFa produced from macrophages and NK cells can maximally activated INF y [19].

The present result showed that the serum levels of TNF a in control positive animals were higher than those of control negative group ,these result may indicated these cytokines play role in inflammatory response against B.abortus infection and in clearance of these pathogens , these evidence was agreement with [20] who investigated that the important cytokines play role in scavenge Brucella consist from TNF a,IL six, INFy and IL 12.INF y and the TNF a secreted from NK cells can activated macrophages to kill Brucella . INF y,1L 12 and TNFa play role in control Brucella infection [20]. the role of TNF a was dependent on presence of INF y in early infection in which it activated macrophages during acute and early chronic phase [21]. INFy is rise in serum of infected mice at acute phase but less amount during chronic phase [22]. while IL six reached peak at acute phase and remain high at first 2 weeks of chronic phase [23] also in the immunized animals at middle chronic phase post infection. The present finding revealed high levels of serum IL six in immunized animals as compared with other groups, these result also indicated that formalin killed Brucella. antigen can elicited IL six that play role in resistance, due to Brucellaabortus is intracellular pathogen in which cell mediated immune response play role in killing these pathogen, the current idea was agreement with Zaitseva et al.[24] who revealed that immunized animals with heat killed B.abortuselicited strong TH1 response against infected by these pathogen,also[25], demonstrated that CD4 Th1 immune response play a major role in the protective host against Brucellaabortus ,however, high levels of IL six in immunized animals in the present study may indicated that activated CD4 Th1 cells stimulated production these cytokines ,these evidence was in consistent with [26].who found that CD4T cells can promote production of IL six.CD 4 T helper play important role in the protective immunity against Brucella infection in addition to cytotoxic T cells [27].

The rseults in current study showed that high levels of serum IL 4 in immunized animals as compared with control positive group but lower than levels of TNF a,, these result may gave indication that IL 4 may play role in immune response against Brucella infection in which both arms of immune responses can play role in protective host against these pathogens ,these idea was agreement with [28] who demonstrated that both type of immune response CMI and antibody play effective role against *Brucella* but CMI are more efficiency in protective host against intracellular pathogens [28] however, TH1 cells involved in the DTH and in CMT while TH2 cells can promote B cells to proliferation and differentiation into plasma production antibodies ,TH1 cells produced INF y,TNFa,IL 2 that mediated the immune related cytotoxic activity a,local inflammation and assist in antibody production ,also all above idea were agreement with present result due to the IL 4 play role in differentiation of Th0 to Th2 cells which produce additional IL 4.[29] that activated B cell and T cells proliferation and differentiation of B cells into plasma producing antibodies as well as decrease development of Th1 cells [30] also it play role in activated alternative macrophages M2 and inhibit classical macrophages and induced decline in the inflammatory reaction [31].

We concluded that killed *Brucellaabortous* antigen can stimulated good cell mediated immune response against *Brucellaabortous* infection of mice.

Conflict of Interest: Non

Source of Funding : Self

Ethical Clearance: Non

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Study of CD69 upon T-cells for SLE Iraqi Patients

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Abstract

Systemic lupus erythematosus (SLE) represented one of the chronic auto-immune multi systemic diseases affecting females more than males especially during childbearing age. A fluctuating nature of SLE disease activity deprive this disease from of any monitoring test, at likewise both of SLE disease activity index (SLEDAI) criteria or even the laboratory analyses do not confirm the disease activity, especially when a patient have normal serological results although during the disease activity (flare). This study done as a case control study, whereas the total sample size included 86 peripheral blood samples, which subdivided into: first the disease group comprised from 68 blood samples voluntary obtained from SLE patients. while the second group included 18 blood samples obtained from healthy individuals (controls) who matched in gender and age with the disease group. however, the disease group were be divided into active group (flare) and inactive group (remission) according to SLE disease activity index criteria by a specialized consultant. This study based on an immunological evaluation of CD69/CD3+ percentages of peripheral blood T-lymphocytes tested as SLE disease activity monitoring marker, owing to that purpose the CD69/CD3+ markers were be isolated from freshly blood T-lymphocytes. moreover in order to getting the best precise for Cluster Differentiation markers percentages CD69therefore were analyzed by Immunophenotyping flow cytometer of multi-color immunofluorescent staining. Whereas the CD69/CD3+ marker ratio can be obtained from data analysis (CY flowsoftware). such ratio set up CD69test as Comparison with SLEDAI criteria, as well as with age and gender factors. whereas CD69% described as early activation marker for T-lymphocytes, so tested for such aim. the results were gave insurance about over expression of CD69T-cells for the active (flare) group. Moreover the most important indicative parameter for disease activity that systemic lupus erythematosus disease activity index whereas CD69/CD3+% has a positive correlation matching with SLEDAI score for SLE disease groups,. Through these results about CD69 which proved as milestoneof SLE monitoring disease activity as independent parameter rather than SLEDAI criteria. Ultimately such results rewarded this study by finding cut off value for SLE disease activity described as CD69/CD3+ SLE activity≥ 21.5000%, within sensitivity71% and specificity 91.7% according to receiver operating characteristic curve. This study proven own aims through of the test named as CD69/CD3+ ratio marker that can be done as more easy, simplest, and accurate singular test instead of various decentralized a huge number of the clinical and laboratory investigations.

Key words: *CD69 upon T-cells, SLEDAI, Flowcytometre, CD3, SLE Iraqi patients.*

Introduction

Systemic lupus erythematosus defined as one of a complex, chronic, inflammatory autoimmune disease [(1)]. That has fickle manifestations follows a relapsing and remitting course. More than 90% of SLE cases affects females [(2)], predominantly at child bearing age [(3)]; with ratio 8:1female –per-male [(4)]. Characterized by impairment within tolerance to the self-antigen and formation of many different auto-antibodies [(5)],as well as the gender especially womenaffected at child-bearing age [(6)]. The chronic sterile inflammation and tissue destruction all these events occur as a result of auto-antigen responsiveness at (SLE) disease mechanisms [(6)]. Thus, T-lymphocytes have a positive correlation with (SLE) Disease Activity Index (SLEDAI) score [(7)]. The

T-cell receptor (TCR) is a complex of total membrane proteins essentially contribute in T-cells activation as a response to induced antigen, such antigens presented in the context of the MHC molecule by APCs. The probability of the increasing CD 69% on T-lymphocytes can be as pure reflection of the increasing CD3 popularity in SLEpatients [8].Already CD69 was described as earliest induced marker on the T-lymphocyte surface after TCR/CD3 complex activation, this expression and appearance of CD69 on plasma membrane of the activated lymphocytes [9]. The expression of CD69 on T-cells surfaces at peripheral blood may give novel insight indicator about monitoring of early activated T-cells population leading to level of T-cell mediated immune responses can also be expected in the body [10].

Materials and Method

-Study population: Whole samples population of such study were 86 individual samples subdivided into first part Disease group that included Active group and Inactive group their samples obtained from both of In-patients and out-patients whom attended AL-Yarmouk Teaching Hospital. As well as patients who attended Al- Al-Karama teaching hospital. and second part of the study wasHealthy group obtained from selected 18 healthy individuals. During the period between October/2017 to June/2018.

(a) Disease group:it wassubdivided according to SLEDAI into:

(a1) Active group (disease flare): The samples were being obtained from 32 patients; 1male and 31females with aging ranged13-60years systemic lupus erythematosus (SLE) patients diagnosed according to disease activity assessed with SLEDAIby specialized physicians.

(a2) Inactive group (SLE remission): these Samples were obtained from 36 patients; 3 males /33 females with aging ranged 13-60years diagnosed by the specialized physicians as SLE patients within remission status according to (SLEDAI).

(b) Healthy control group: The samples were being obtained from the 18 healthy Individuals 2 males, 16 females their age ranged 13-60years volunteer after take their conceptions.

Samples collection: All samples were be collected at the same manner from each individual, which described as 2.5ml of whole venous bloodhave been collected under sterile conditions then it was poured into heparin tube for CD3 & CD69 flow cytometric analyses.

The preparedantibodies againstCD3 marker was mouse IgG1 conjugated with FITCwhile suchprepared antibodiesagainst CD69 marker were mouse IgG1 conjugated with R-PE, Ultimately both markers were be diagnosed by BD FACs™ brand flow cytometer.

Flow cytometer: one improved table flow cytometer (Cy Flow) instrument used for detecting and analyzing of T-lymphocytes CD3 and CD69 markers, that must be equipped for appropriate fluorescence laser excitation at 405nm, 488nm, and 635nm with appropriate software (Cy View) software for data acquisition as well as analysis.

Flow- cytometer kits: I- CD3(SK7) monoclonal mouse anti-human, **II-**PE Mouse Anti-Human CD69Becton, Dickinson and company BD Bioscience, BD Bioscience Pharmingen™/USA.

Procedure:20 µl were used for both of CD3 fluorochrome- conjugated monoclonal- antibody and CD69 fluorochrome-conjugated monoclonal antibody then were added to 100µl of freshly whole blood in 12x75-mm capped polystyrene test tube;at the sametime. The tested sample was thoroughly vortexthenmustbe incubated 15-30 minutes in the dark place at room temperature 20°C-25°C.Anyway about 2µl of 1x BD FACS lysine solution also were added after incubation, then thoroughly vortex and incubated for 10 minutes in the dark place at 20°C-25°C. whereas tested sample was centrifuged at 300g for 5minutes. Washing 3times by adding buffer solution, yet supernatant were discharged. Finally2µl from (FITC) solution were addedto thesample. the samples were tested byPartec Cy Flow® instrument; then the tested results were displayed on data show within a special program named as (Cy View software).

Statistical analysis: Analyses of data were carried out using the available statistical package of SPSS-24. Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range. The Students-t-test was used for difference between two independent means or Paired-t-test for difference of paired observations or ANOVA test for difference among more than two independent means. Statistical significance was considered whenever P value ≤ 0.05[11].

Findings

Frequency of CD69/CD3^{+}T-cells % Expression among this study groups

The distribution of such study groups displayed on Figure 1 explained the distribution of tested samples within each group according to CD69/CD3^{+}% of peripheral blood.

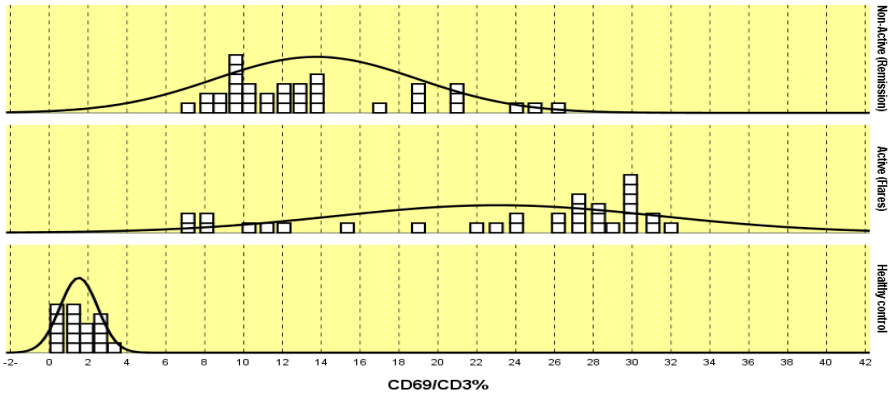

Figure1: Distribution of CD69/CD3^{+} ratio means± SD for studied groups within lighting highly significant differences among studied groups.

Comparison ofCD69/CD3^{+} frequency for the studied groups according to age and gender

Table1 shows the frequency of distributed groups according to age and gender with CD69 T-cells mean ±SD as a monitoring marker for SLE disease activity. the most highly significant means and standard divisions with age group <30 years for active group mean ±SD=26.80±0.34, likewise inactive group mean ±SD=11.56±1.83, finally healthy group mean ±SD=1.73±1.47. the tested marker CD69/CD3^{+} % pointed mean differences between male/female for each studied groups with highly significant differences p value 0.0001, P≤ 0.05.

Table1: CD69/CD3^{+}% frequency among studied groups as comparisons depending on age & gender

Parameters Active (Flares) Mean ±SD (Range)		**CD69/CD3^{+}%**			
		Non-Active (Remission)	**Healthy control**	**P- value**	
		Mean ±SD (Range)	**Mean ±SD (Range)**		
Age (years)	<30	26.80±0.34 (26.41-27.0)	11.56±1.83 (9.86-13.50)	1.73±1.47 (0.47-3.34)	0.0001#
	30--39	25.57±3.91 (19.0-30.0)	11.98±3.32 (6.95-19.0)	1.31±0.46 (0.92-1.82)	0.0001#
	40--49	20.01±9.84 (7.45-32.0)	13.80±5.61 (8.39-26.0)	1.52±1.21 (0.35-2.91)	0.0001#
	=>50	25.60±9.36 (6.83-31.14)	16.32±6.41 (8.36-25.0)	1.52±0.78 (0.25-2.38)	0.0001#
Gender	Male♂	25.34±	15.96±5.63 (9.89-21.0)	1.63±0.07 (1.58-1.68)	0.083
	Female♀	23.33±8.39 (6.83-32.00)	13.53±5.21 (6.95-26.0)	1.50±1.04 (0.25-3.34	0.0001#
*Significant difference between two independent means using Students-t-test at 0.05 level.					
#Significant difference among more than two independent means using ANOVA test at 0.05 level.					

CD69/CD3^{+}T-cells ratio frequency among studied groups according to SLE disease activity index scores

the correlation between CD69/CD3^{+}%T-cells with SLEDAI scores showed no shared points among the studied groups which displayed on Figure 2 follows:

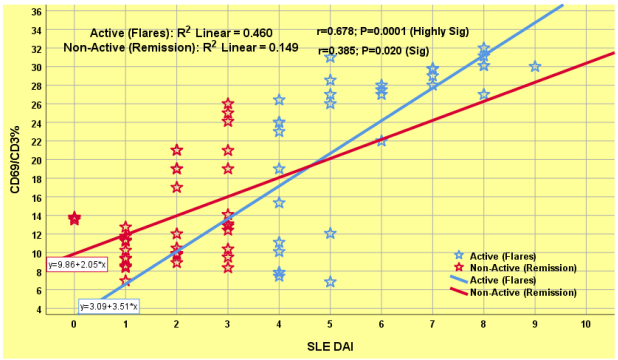

Figure 2 : Linear Regression of CD69/CD3^{+} depending on SLEDAI scores for each of SLE active group that indicated highly significant positive correlation while SLE in active g group low significant positive correlation.

Speculation sensitivity and specificity for CD69/CD3+T-lymphocytes of peripheral blood ratio marker according to ROC curve

According to Receiver Operating Characteristic (Roc) curve CD69/CD3+ ratio giving sensitivity 71.9%while marked specificity 91.7% both in at SLE flare group CD69/CD3+ ≥ 21.5000 % which considered as cut off SLE disease activity ratio.

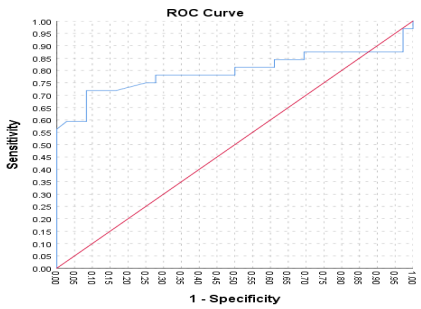

Figure 3: Receiver Operating Characteristic (ROC) curve indicating fundamental area under CD69/CD3+ curve.

Discussion

This study detects SLE disease age ranged from 21to 59 years among samples of SLE disease group patients. These results agreed with Al-Haidary was records the peak range of SLE Iraqi patients from20to69 years[12], while the range 14-61 years was recorded by Al-Saady [13], and Noori *et al*. reported the mean age of Iraqi SLE patients was±SD=27.8± 10.7[14]. also, This study results corresponds with Arabic researches about SLE disease like Al Dhanhani *et al*. who reported the Arab United Emirates mean age was ±SD=28.6± 12.4 years[15]. Moreover Ku *et al*. referred to range of Chine's' women age was 9-40[16]. actuallythis results agreed with Noori *et al*. who reported the percentages of the males 2.0% , and females 98% [14].these results pointed thecut off scorefor diseaseactivity was ≥4score. Whereas inactive group patients pointed at less than or equal 3 score ≤3 according to this study. However Bonelli *et al*. who reporting that the score of their patients was higher than usual score because they were have lupus nephritis ≥6 score, but also they reported about all previous studies opinions which considered the lower cut off disease activity at >3[17]. Compagno who reported there was a significant correlation between SLEDAI score and lupus nephritis[18]. Furthermore this study provides the percentagesbout some Iraqi SLE patients depending on SLEDAI scores. However there is no Iraqi study matched with SLEDAI scores% results. the current pointed highly significant correlation with SLE disease activity among groups under study depending on CD69/CD3^{+}T-cells ratio as immunological markerthat played an early inducible marker for T-lymphocytes activation and migration according to Radulovic *et al*. [19]. our results approved with Martin *et al*. who ensured that CD69 considered as early activation receptor within an intrinsic differentiation program where determined immune inflammatory process in SLE patients [20]. Whereas the previous researchers results corresponds with the current study, like Su *et al*. and Syh.Jae *et al*.[10,21].Finally, the previous researchers supported CD69/CD3+ T-cells % as accurate independent marker for SLE disease activity assessment.Also about American females SLE disease activity age ranged 20-30 years by Weckerle and Niewold [22]. Moreover Ku *et al*.claimed that optimum activity age was 28± 4.96 years[16]. Furthermore, although that SLE disease mostly effects females than males, which may be due to immune system differences by Weckerle and Niewold [22],Actually, SLE disease activity index consider as an essential requisite for CD69/CD3+% T-cells evaluation test whereas successfully signify highly correlation with its scores reached to mean ±SD >30± % at 9 score. also disease activity was assessed by measuring SLEDAI scores, the weighted average of SLEDI scores>10" by Kakati *et al*.[23]. So according to SLEDAI scores selection process done accordingto the patients of this study within scores lower than average >10 score in order to investigate the early activation process prior disease flare.Ultimately the ROC test was

gave cut off disease activity value ≥ 21.5000% for CD69/CD3$^+$%. This study suggested that CD69/CD3$^+$ratio can be considered as immunological laboratory test for SLE disease activity control as independent monitoring test rather than SLEDAI criteria. this study was deemed as the first study seeking about CD69/CD3$^+$ T-cells of peripheral blood for Iraqi SLE patients by using flow cytometer which detects cut off SLE disease activity. the conclusion: There is highly significant correlation between CD69ratio with age 21-59 years for female gender.this marker CD69/CD3$^+$% T lymphocytes of peripheral blood may be considered as accurate singular independent monitoring test for SLE disease activity (flare) within cut off value ≥ 22.5%.

Conflict of Interest: Non

Source of Finding: Self

Ethical Clearness: Non

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Effect of *Fusariumgraminarum* Silver Nanoparticles on IL-10 and IFN-γ cytokines in Mice after Infected by *Leishmaniadonovani*

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Abstract

Leishmaniasis remains one of the fatal diseases worldwide, and the conventional antileishmanial therapies are toxic and most are expensive. Biological silver nanoparticles possess broad-spectrum antimicrobial activities and could be a future alternative to current antimicrobial agents. In the present study an approach was made to synthesize silver nanoparticles using a *Fusariumgraminarum* fungus.To investigates the efficiency of silver nanoparticles against ***Leishmaniadonovani***compared with pentostam drug *in vivo* by measuring the levels of immune cytokines (IL-10 and IFN-γ) in serum infected mice and treated with AgNPs (0.1 ml / day) and comparisons with pentostam drug (0.01 / day) after 7,14 and 21 days of treatment. The results showed that the level of IFN-γ in grouptreated with AgNPs increased significantly in third weeks, compared withpentostamgroup.While treated the infected mice withpentostam/AgNPs together, gradualdecreaseoccured in the level of IFN-γ in all groups, compared with negative control. Also a significant increaseoccurs in the IL-10 level within 21days when mice were treated with AgNPs compared with pentostam.

Key word: *Leishmaniadonovani*, ***pentostam, silver nanoparticles, IL-10, IFN-γ***

Introduction

Leishmaniasis is one of the vector-borne diseases caused by obligate protozoan parasites of the genus *Leishmania*, they are transmitted by different species of sand flies belong the genus of *Phlebotomine* as extracellular flagellated promastigotes that replicate as an intracellular parasite (aflagellateamastigotes) in mononuclear cells of mammalian hosts[1]. Visceral leishmaniasis is considered as the second cause of mortality and the fourth cause of morbidity after malaria, schistosomiasis and African trypanosomiasis[2]. Pentavalentantimonials are a group of compounds used for the treatment of leishmaniasis. The compounds currently available for clinical use are sodium stibogluconate and meglumineantimonate. The Role of cytokines such as IFN-γ is to activate macrophages and enhance the microbicidal activity of these cells to kill intracellular pathogens through the generation of reactive oxygen species and reactive nitrogen species. IL-10 promotes intracellular infection, including human visceral leishmaniasis, by disabling Th1 cell type responses and/or deactivating parasitized tissue macrophages[3].

Nanotechnology continues to attract significant attention due to its impact on many currently important areas such as energy, medicine, electronics and the aerospace industry. Nanoparticles that possess one or more dimensions of the order of 100 nm or less continue to attract significant attention due to their unique properties in the realms of chemistry, optics, electronics and magnetism[4].

The use of eukaryotic organisms such as fungi, *Fusariumgraminarum* and other species offers considerable promise for large-scale metal nanoparticle production since the enzymes that are secreted by the fungi represent an essential ingredient for the biosynthesis of metal silver nanoparticles has attracted high interest

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due to their unique and excellent properties in addition to its therapeutic potential for the treatment of a variety of diseases that includes retinal neovascularization and acquired immunodeficiency syndrome due to human immunodeficiency virus [5].

Materials and Method

Silvernanoparticles (AgNPs) preparation:

Silvernanoparticles (AgNPs) preparation according to *Birla et al*.,l[6]methode**.**

Characterizationof nanoparticles

The exact configuration of the, size, concentration, morphology of crystals, aggregation state and even bioconjugation and was measuredby using particle the following techniques:Atomic force microscopy (AFM) (AA-3000, Angstrom, USA), X-Ray diffraction (XRD) (Shemadzu, Japan), Ultraviolet-visible spectroscopy (UV-VIS)(Shemadzu, Japan)[7].

Parasite strain and culture

Leishmaniadonovani was isolated from the bone marrow of an infected child , the strain was obtained from biotechnology center/ AL- Naharin University, it was cultured and maintained by serial passage in NNN media each 8 days and incubated at 26°C.

***Leishmania* antigen preparation**

One milliliter of promastigote culture in stationary phase washed three times with phosphate buffered saline by centrifuge 4000 rpm in 15 minutes then adjusted to concentration 1× parasite\ml.

Animals

Ninety six Male *albino* mice aged between 8-12 weeks, weighing 20-28 gm was obtained from The National Center for Drug Control and Research, housed under standard condition in animal house in the biology department in College of Science/AL-Mustansrya university . Seventy eight mice were infected with 1×10^7 parasite/ml *L. donovani*promastigotes by injectionintraperitonial[9]. Then the groups were inoculated as a follow:

- Group 1 : inoculated orally by stomach tube (0. 1ml/day) normal saline considers as control positive group.
- Group 2 : inoculated orally by stomach tube AgNPs (0.1ml/day) for 21 days considers as an AgNPs treatment group.
- Group 3: injected with (0.01 ml/day) from Pentostam drug by intramuscular each day for 21 days considers as Pentostam treatment group.
- Group 4 : inoculated orally by stomach tube AgNPs (0.1ml/day) and injected with (0.01ml/ day) from Pentostam drug by intramuscular for 21 days consider as AgNPs and Pentostam treatment group.
- Group 5: inoculated orally by stomach tube (0. 1ml/day) normal saline considers asnegative control without infecting by *L. donovani* parasite..
- **Blood collection and animal anatomy**

In the days 7th, 14th, 21th the blood samples were collected from all experiment, mice from facial vein collected 2 ml of blood in the sterile plane tube, then left at room temperature, after 35min centrifugation the clotting blood, obtained clear serum and stored at -20°C until the examination for immunologic test.

Determination of cytokine levels

The serum levels of IL-10 and IFN-γwere analyzed by ELISA (human Systems, Germany). The cytokines were quantified usingmanufacturer's protocol.

Statistical analysis

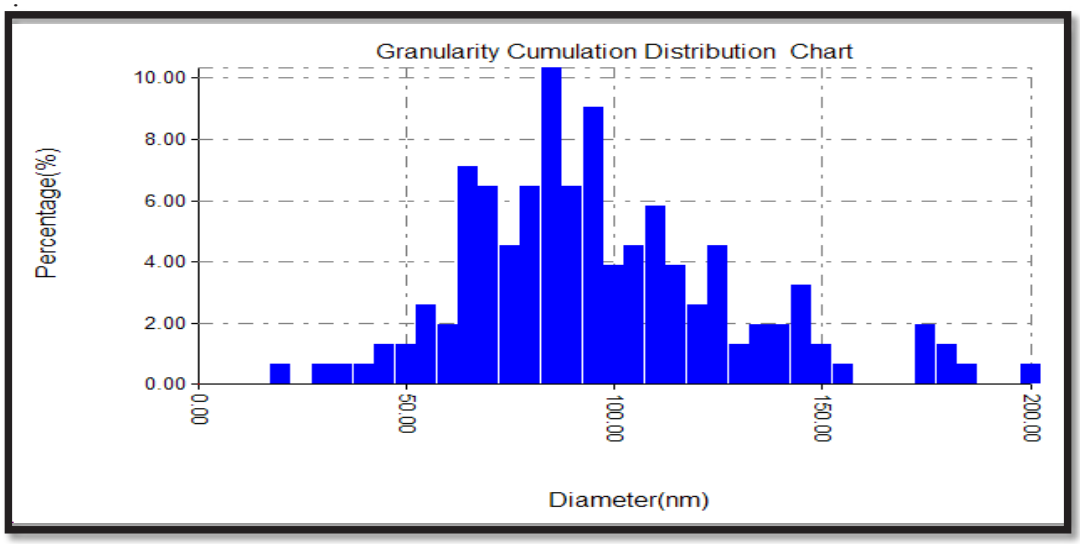
The Statistical Analysis System- SAS (2012) program was used to effect of different factors in study parameters. Least significant difference –LSD test (ANOVA) was used to significant compare between means in this study[9].

Findings

Detection of the existence of *Fusarium*AgNPs

After adding of silver nitrate ($AgNO_3$) to filtered cell, the color of the mixture changed from colorless to blackish brown compared with negative control remain clear (colorless), the changes in color which confirms the reduction of $AgNO_3$ by *F. graminarum*indicated the presence of AgNPs[10].

Morphology of*Fusarium*silver nanoparticles by atomic forcemicroscopy Determine *Fusarium* silver nanoparticles (AgNPs) sizes and surface morphology were measured ,using the software of the AFM , the images of AFM for *Fusarium*AgNPs in fig. (1)represents particle size distribution, where average diameter is 94 nm. While in fig.(2A,B) is AFM picture in three dimensions (3D) and two dimensions (2D), it explains structural shape for grains,found that the average roughness (Ra) is 9.33 nm and Root mean square (Sq) is 11.6 nm.



Figure(1):Granularity volume distribution chart of silver nanoparticles produced by *F. graminurum*

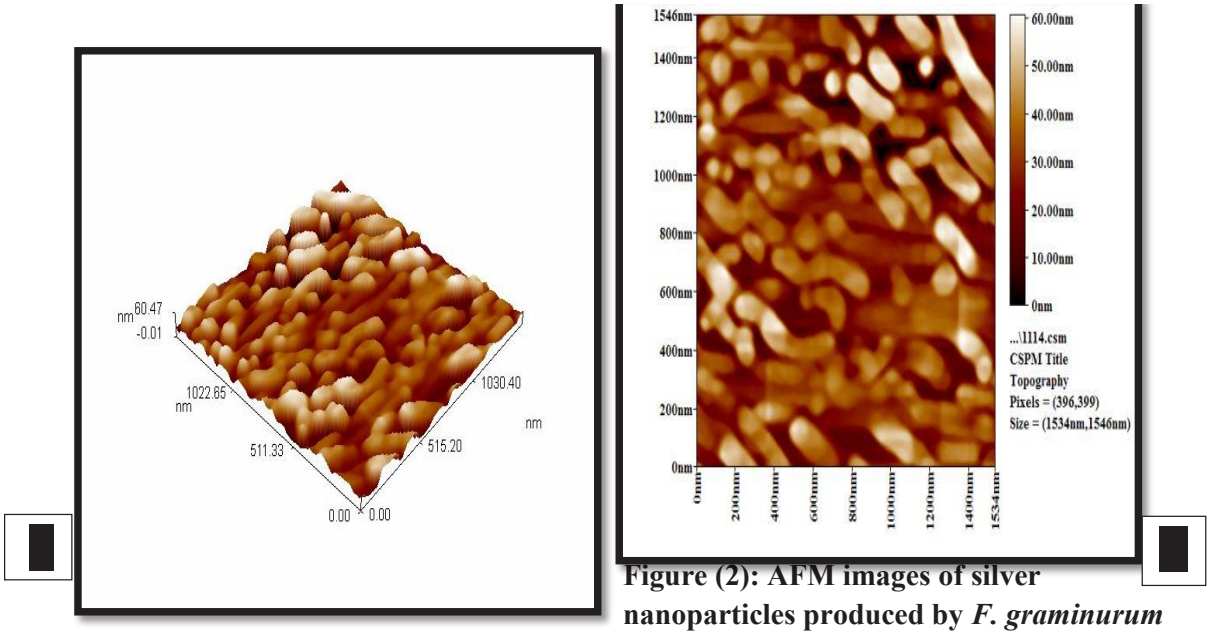


Figure (2): AFM images of silver nanoparticles produced by *F. graminurum* (A) three dimensions 3D and (B) two dimensions 2D

Characterization of *Fusarium*AgNPs by X-RayDiffraction

A typical XRD pattern of *Fusarium*AgNPs as shown in fig.(3), the diffraction peaks at 38.05°, 44.22°, 64.32 and 77.31° were correspond to the (111), (200), (220) and (311) facets of the face centered cubic crystal structure, therefore the average crystallite size was 28.225 nm.

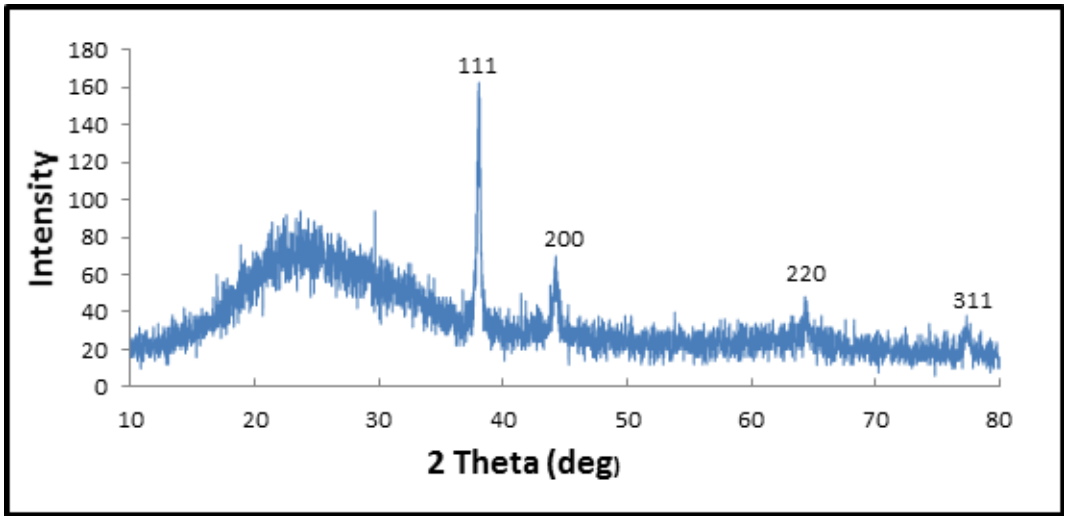


Figure (3): X-Ray pattern of silver nanoparticles produced by *F. graminurum*.

Optical properties of*Fusarium*AgNPs by UV–Visible Spectral:

This technique confirms the presence of *Fusarium*AgNPs by measuring the absorbance of the bioreduced solution at wavelengths between 300 and 800 nm. Extinction spectroscopy of ultraviolet (UV) and visible (Vis) light (UV–Vis spectrum) allows confirming the presence of *Fusarium*AgNPs because of the characteristic plasmon resonance, which showed an absorbance peak at 420 nm, fig (4)..**Figure (4):UV-Visible spectroscopy of silver nanoparticles produced by *F. graminurum***

Determine sera level of cytokines in mice serum

Serum level of Interferon-γ (INF-γ)

After mice are infected with *leishmania* parasite, the level of INF-γ was increased in the three weeks and after treated of these mice with pentostam and *Fusarium*AgNPs observed that the level of INF-γ has increased in all groups compared with the negative control group, shown in table (1) significant differences ($P<0.01$) between all groups compared with control .In control positive group the level of cytokine was elevated from the first week was (183 ± 13.44pg/ml). While the pentostam group was observed to increase occurredin the first week (305.17 ± 40.76 pg/ml). InAgNPs group the level of INF-γ has increased gradually over the three weeks (284.17 ± 42.97Pg/ml , 303.83 ± 51.98Pg/ml and 410.00 ± 10.52Pg/ml)respectively. In P/AgNPsgroup gradual decrease occurred since the first week (284.17 ± 42.97 pg/ml) ,and gradually continues to declinein second and third weeks (218 ± 36.63 pg/ml and169.50 ± 8.41 pg/ml) respectively .

Table 1.The serum level of INF-γin the experimental groups after three weeks (Mean ± SD)

The Groups	Time (day)			LSD value
	7	14	21	
Control(–ve)	115.67 ± 4.80	118.33 ± 5.04	123.33 ± 1.63	18.542 NS
Control(+ve)	183 ± 13.44	239.17 ± 4.35	323.17 ± 14.79	31.602 **
Pentostam	305.17 ± 40.76	301.83 ± 49.94	315.67 ± 61.46	26.524 NS
AgNPs	122.50 ± 8.87	303.83 ± 51.98	410.00 ± 10.52	37.277 **
P/AgNPs	284.17 ± 42.97	218.00 ± 36.63	169.50 ± 8.41	31.842 **
LSD value	42.668 **	47.073 **	61.905 **	---

****a significant difference at P<0.01 , NS: Non-Significant.**

Means having with the different small letters in same column differed significantly.

Serum level of Interleukin–10 (IL-10)

The serum levels of IL-10 in treated groups of the present study were high level in groupspentostam and *Fusarium*AgNPs infected with *leshmania*parasite were (338 ± 16.07 and 467.83 ± 34.43)respectively in the third week. While the level of IL-10 in control positive group was lower level (320.67 ± 6.37 pg/ml) in third weeks compared with other control negative group was (97.67 ± 6.62 pg/ml). As well as in the P/AgNPs group also elevated, but less than other treated groups in the third week (336.17 ± 22.06 pg/ml) as illustrated in table (2). There was significantly different ($p \leq 0.01$) between all groups compared with positive control.

Table 2.The serum level of IL-10in the experimental groups after three weeks (Mean ± SD)

Groups	Time (day)			LSD value
	7	14	21	
Control(-ve)	95.17 ± 2.31	96.33 ± 1.97	97.67 ± 6.62	11.547 NS
Control(ve+)	178.50 ± 8.87	232.50 ± 5.89	320.67 ± 6.37	28.761 **
Pentostam	290.83 ± 23.31	451 ±20.53	338.00 ± 16.07	32.804 **
AgNPs	112 ± 8.09	456 ±26.61	467.83 ± 34.43	41.952 **
P/AgNPs	121.83 ± 7.46	141.50 ± 2.88	336.17 ± 22.06	30.776 **
LSD value	27.336 **	42.602 **	41.735 **	---

**** mean significant difference at P<0.01 , NS: Non-Significant.**

Means having with the different small letters in same column differed significantly.

Disscusion

The synthesis of silver particles by using *Fusariumgraminarum*was observed during change the color of the mixture from colorless to blackish brown, the color change confirmed the formation of nanoparticles,these results corresponding withBawaskar*et al.*[11], Mahmoud *et al*[11] .

This study, the results are near to results of Shafiq*et al*.[12].showed that, the XRD diffraction measured in Ag-NPs resulted in four intense peaks and this further confirms that the Ag-NPs formed in the extracellular filtrate are present in the form silver nanocrystals. Vijayan*etal.*[13] observed that AFM topology is very helpful in revealing the exact size and shape of silver nanoparticles.Jebali and Kazemi[14]reported that highest anti-leishmanial drug was observed for Ag NPs, and AuNPs were in the second level, as well as they found that anti-leishmanial activity of metal nanoparticles is higher than metal oxide nanoparticles that may be due to oxidation capacity of metal nanoparticles, leading to moredamage to membranes, enzymes and DNA.

The results of cytokine indicated an increase occurs in serum level of IL-10 and INF-γduring three weeks post-infection as showed by Kamil*et al*.[15]reported a significantly increased serum level of IFN-γ and IL-10 in VL patients compared to healthy controls, also confirmed by Khoshdel*et a.l*[17] reported thatthe levels of the serum cytokines, IL-10, IL12, and IFN-γ were higher in patients than in family members and control individuals.

Also, in a previous studydemonstrate the elevation of both IFN-γand IL-10 mRNA levels in the lesional environment of thebone marrow in patients with kala-azar before therapy, findings which may be of importance in understanding how thisorganism is able to avoid immune-mediated destruction by itshost macrophages[17].Inactive visceral leishmaniasis, Al-Autabbi*et al.*[18]reported that the immune system is highly activated and produce both the macrophage-activating cytokines IFN-γ and the macrophage-deactivating cytokines IL-10.

IL-10 is able to inhibit Th1 cell and macrophage activation; therefore, higher levels in the sera of VL might be expected, IL-10 also plays an important role in regulation of inflammatory response, and is important for the survival and persistence of the parasite inside macrophages [19]. In other reports showed that, the data favor a role for IL-10 in conditioning the host cells so that they become poorly responsive to even high levels of IFN-γ for intracellular killing [20].

IFN-γ plays an essential role in macrophage-mediated antileishmanial activity, contributing to parasite elimination and the subsequent resolution of infection [21]. Also, IFN-γ cytokine is the main factor in inducing the transcription of inducible nitric oxide synthase (iNOS) and the production of nitric oxide (NO) [22].

Nanoparticles can undergo a series of processes, including binding and reacting with proteins, phagocytosis, deposition, clearance, and translocation. At the same time, nanoparticles can elicit a spectrum of tissue responses, such as cell activation, generation of reactive oxygen species, inflammation, and cell death[23]. A significant increase in the level of IFN-γ was detected in the serum of patients during treatment with pentostam when compared to its level before treatment, this explains that a successful drug therapy were restored T-cell proliferation and IL-2, IFN-γ production in response to *Leishmania*antigen [24].

Conclusion

Silver nanoparticles synthesis from *Fusariumgraminarum*is safety, nontoxic andit can be considered as a new antileishmanial agent. Also*Fusarium*AgNPslead to induce pro and anti-inflammatory cytokines.

Conflict of Interest: Non

Source of Funding- Self

Ethical Clearance: we have got admission from biotechnology center/ AL- Naharin University and the biology department in College of Science/AL-Mustansryauniversity.

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Antifungal Activity of *Boswellia serrata* Gum Extracts on Antifungal Drugs Resistant *Aspergillusfumigatus* Isolated From Diyala Patients

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Abstract

This study focused on antifungal activity of alcoholic extracts of *Boswellia* gum onthe potent antifungal resistant opportunistic pathogen for humans, *Aspergillus fumigatus*. The antifungals used were: Terbinafine, Nystatin and Clotrimazol.Antimicrobial susceptibility testingof alcoholic gum extracts of *Boswellia* was done by well diffusion methodagainst the mold. Results showed that Boswellia extracts had the ability to inhibit fungal growth *in vitro* and the statistics revealed different inhibition zones gradually increased with the increasing in the concentration of theextract and that acetonic extract was more effective than ethanolic. Confirming the presence of saponins,volatile oils,terpensand phenolic compounds in acetonic extract withphenolic compounds in ethanolic extract.

Key words: *Aspergillus fumigatus, Antifungal resistance, Boswellia extract*

Introduction

Aspergillus is an important pathogen of humans and animals,it is a leading cause of human fungal ailments with *A. fumigatus* as the main causative agent of invasive fungal diseases. *A. fumigatus*[1]. Aspergillosis is the name given to a wide variety of diseases caused by infection by this fungus, the majority of cases occur in people with tuberculosisor Chronic Obstructive Pulmonary Disease [2], but with otherwise healthyimmune systems[3][4].It is estimated that otitis externa make up 9 to 25% are caused by fungi, calledfungal otitis or otomycosis[5][6].However, antifungal resistance occurrence has to be considered independently for each antifungal class, fungal genus and epidemiological data regarding incidence of distributed worldwide [7].Despite improvement of antifungal therapies over the last 30 years, the phenomenon of antifungal resistance is still of major concern in clinical practice[8].Since the thousands of years, people used to take plant originated products and the natural extracts prepared from various species of *Boswellia* tree for the treatment of several diseases[9][10].Many previous studies indicated itsbiological therapeutic effectssuch as inflammation and antioxidant[11][12][13],anticancer[14] and antimicrobial[15].

Many of studies indicated its significant antifungal properties against toxigenic Aspergillii [16][17].We need the medicinal plants as antifungal agents instead of using artificial drugs which have several toxic and side effects. Therefore, the objective of this study was to estimate antifungal potential of *Boswellia* against selected mould species.

Materials and Method

Preparation of Plant extract: The gum resinsof *B. serrata*waspurchased from local market ofBaqubah, Diyala, Iraq. The extraction was prepared according to Matny*et al*[18] with slight modifications using Lyophilizing technique. The two extractswere stored in a well-closed containers, protected from light and kept at 4°C until use.

Phytochemical analysis: Screening of most secondary metabolites in the crude extracts of*B. serrata*was determined as following:

- Test for alkaloids and terpens was performed according to Njoku and Obi [19].
- Test for saponins, tannins and glycosides was

performed according to*Obasi et al*[20].

- Test for phenolic compoundswas carried out according to the method described byHarborne[21].
- Test for volatile oilsperformed according to Indian Herbal Pharmacopoeia[22].

PreparationofPlantExtractConcentrations: Stock solutions (200mg ml^{-1}) was prepared by mixing 2 g of the dried extract with 10 ml ethylene glycol50% (solvent used as diluents solution), and then it was sterilized with millipore filter membrane (0.22 µm),then different concentrations of(100,50)mg ml^{-1}were prepared by mixing known volume from the stock solution with ethylene glycol.

Antifungal drugs Preparation: The commercially available samples of antifungal drugswere:Terbinafine, Nystatin, and Clotrimazol. Each antifungal was prepared as an initial concentration ‘’working stock solution’’ of 10 mg ml^{-1}[23]

Preparation of tested microorganism: Twenty samples were collected from patients with Aspergillosis Otomycosis in Baqubah Teaching Hospital (Diyala Governorate). The samples were 8 of sputum, 2 nose swabs and 10 ear swabs. All specimens were collected from January to April 2018. The samples were examined directly under the light microscope objectives to detect fungi and their septate hyphae [24]. Then culturing on the Sabouraud dextrose agar to observe the macroscopic characteristics and the isolates were identified to the species level on the basis of microscopic characteristics[25] [26][27][28]. Fungal inoculum was prepared by the use of haemocytometer to count fungal conidia[29].

The activity of the antifungal drugs and plant extracts*in vitro*: Antifungal susceptibility testing was done by agar well diffusion method to detect anti-fungal effect for all *A. fumigatus* isolates towardantifungals(Nystatin, Terbinafine and Clotrimazol),andthe extractswere performed against only the antifungal drug resistant isolate. All tests were in triplicateusing the solventethylene glycol [30].

Statistical Analysis: The results were analyzed by using one-way variance analysis (ANOVA). All of our analysis were done by SPSS-18. Differences at $P < 0.05$ were considered statistically significant.

Findings

Physical and PhytochemicalInvestigation: Acetonic extract of *B. serrata* was viscous to colloidal texture with tan to brown color, not soluble in water and the percentage was14% of crude gum resin. While ethanolic extract was powdery texture with pale yellow in color, less soluble in water and the percentage was 6% of crude gum resin. Phytochemical analysis for active compounds was illustrated in table 1.

Isolation and Identification of *A. fumigatus:* Cultural and microscopic examination illustrates that the infections belonged to the fungus *A. fumigatus* in 20 specimens were 13 isolates. Figure (1) show the microscopical and cultural feature of the fungus.

The Sensitivity of *A. fumigatus* o the antifungal drugs*In Vitro*:The isolates were a variable in their sensitivity to drugs (figure 2). However one isolate was resistant to all antifungal drugs.

Antifungal activity of the plant extracts *In Vitro:* As shown in figure 3, the results of statistical analysis showed significant ($P \leq 0.05$**)** inall concentrations (200,100 and 50 mg ml^{-1}) of the acetonic extract of *B. serrata*gum resin which showed inhibitory activity against*A. fumigatus* incomparison to the ethylene glycol (control).While ethanolic extract shows the inhibitory activity at high concentrations (200 and 100 mg ml^{-1}) only with less efficient incomparison to acetonic extract(Figure 4).

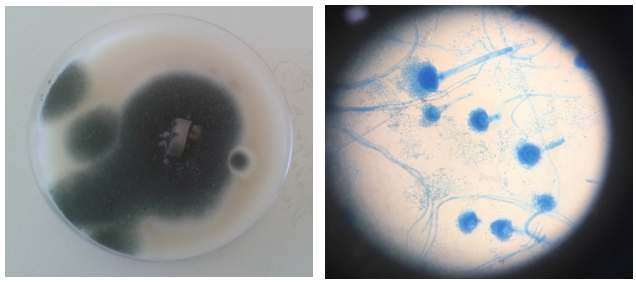

Figure 1: (a) *Aspergillus fumigatus* grown on SDA at 37°C after 7 days of incubation,(b) Microscopic feature of *A. fumigatus* stained with Lactophenol cotton blue.

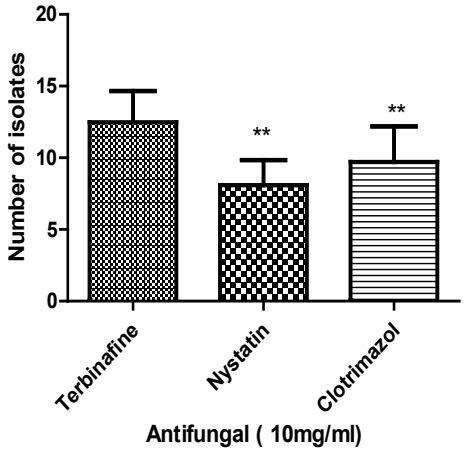

Figure 2: Sensitivity of *Aspergillus fumigatus* to antifungal drugs

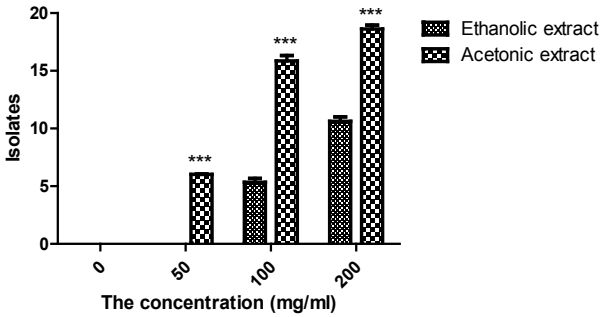

Figure 3: Effect of *Boswellia serrata* gum extracts on *Aspergillus fumigatus*

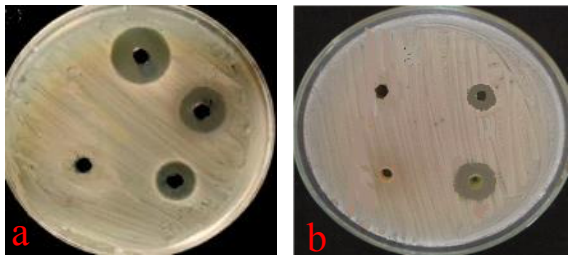

Figure 4: Effect of (a) acetonic and (b) ethanolic extract of *Boswellia serrata* gum on *A. fumigatus at the concentrations(200*,100, 50)mg** ml^{-1}**(1,2,3), C: control (ethylene glycol).**

Table. (1): Phytochemical analysis tests of *Boswellia serrata* gum alcoholic extracts

Phytochemical Constituents	Result	
	Acetonic extract	Ethanolic extract
Alkaloids	Negative	Negative
Saponins	Positive	Negative
Tannins	Negative	Negative
Glycosides	Negative	Negative
Terpens	Positive	Negative
Phenolic compounds	Positive	Positive
Volatile oils	Positive	Negative

Discussion

Phytochemical screening of many active materials of *B. serrata*acetonic extract showed positive. While, ethanolic extract was positive for phenolics only(Table 1).Many of studies were made about the chemical composition of this plant. In 2009,Upaganlawar and Ghule[31]isolated boswellic acid and many of terpenoids. Al-Ogaili *et al*[15], showed positive results for saponins and negative for phenolic compounds in on aqueous extract. Another studies showed that resins chiefly contain higher terpenoids which are mainly the former of its pharmacological effects [9][32].Methanolic and ethyl acetate extract of this plant showed the presence of tannins, phenols, steroids, flavonoids, and saponins [33].Venkatesh *et al*[34]extracted the essential oil from this plant. Study the plant extracts by polar solvents such as alcohol (acetone or ethanol) provided more consistent antifungal activity compared to those extracted by water; this might have resulted from the lack of solubility of the active constituents in aqueous solutions[35]. Further trials using solvents of various polarities will explore the effects of solvent composition on extract efficacy. While, alcohol extract showed some degree of antifungal activity. Therefore, Ethylene Glycol used in this study as a solvent, it is chemically colorless, odorless and possessed sweet taste alcohol used as solvent or co-solvent offers wide range of advantages such as; biocompatibility, biodegradable, stable, hygroscopic, non-toxic and more importantly water-solubility, it possess fungistatic properties thus acts as preservative [34]. In the present study, Ethylene glycol solution was used as a solvent for crude alcoholic extracts and to investigate the inhibitory activity of the crude extracts against antifungal drugs resistant *A. fumigatus*.

Aspergillus fumigatus biofilm associated infections are emerging as life-threatening infection with a very high mortality rate even after antimycotic therapy [36]. In spite of the extensive research dedicated to the

development of new therapeutic strategies, there are only a limited number of available drugs to fight against invasive fungal infections[37]. In this study, the effect of three antifungal agents (Terbinafine, Nystatin and Clotrimazol) on *A. fumigatus* was illustrated in (figure 2). Statistical analysis had shown significant difference at a level of probability P (< 0.05) between antifungal drugs and *A. fumigatus* isolates. This result was agreed with Brinboum [38]who concluded that Terbinafine was characterized by its impact on enzymatic activity of pathogenic fungi.Mechanisms leading to Clotrimazol (azole) resistance may be decrease in azole affinity for their target, increase in azole target copy number, alteration of ergosterol biosynthetic pathway after azoles action, and decrease in intracellular azole accumulation [37]. In this study, by increasing the concentration of alcoholic extract, inhibition zone around the well was increased (Figure 3 and 4). Antifungal activity of *B. serrata* may be due to the active components which are present in plant gum extracts (Table 1);Sharma *et al*[33] found that methanolic and ethyl acetate extract showed good antifungal activity against *A. niger* with a zone of inhibition 20 to 22 mm.Venkatesh *et al*[34]showed that the essential oil of *B. serrata* had fungicide activity against *A.fumigatus* at MIC (0.15 µg ml^{-1}) and MFC (5 µg ml^{-1}). Cowan[39] showed the mechanism that thought to be responsible for toxicity of plants active materials to microorganisms, he suggested that phenolic compounds mechanism includes enzyme inhibition by the oxidized compoundsthrough reaction with sulfhydryl groups or through more nonspecific interactions with the proteins and there is also evidence for direct inactivation of microorganisms: low tannin concentrations modify the morphology of germ tubes of fungi.

Conclusion

The present study demonstrated that the acetone extract of *B. serrata* hold an excellent potential as an antifungal agent. Further *in vivo* studies are necessary. More importantly there is need for detailed scientific studyto investigate other active compound in addition to essential oils of *B. serrata* that affects in the studied mold.

Conflict of Interest and Authorship Conformation: All authors have participated in analysis and interpretation of the data; they drafting the article or revising it critically for important intellectual content. And finally, this manuscript has not been submitted to another journal.

Source of Funded: This research is self-funded and the authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

Ethical Clearance: We undertake to follow the ethical principles and institutional requirements guiding (Baqubah hospital), this research and ensure that they sign the personal declaration of responsibility prior to their isolates involvement in the research.

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Prevalence of β-lactam ResistantKlebsiellaPneumoniaeIsolates among Clinical Specimensin BaghdadHospitals

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Abstract

One hundred forty three of *Klebsiellapneumoniae* isolates had been collected from some hospitals in Baghdad city. The isolates were taken from different clinical specimens.Antimicrobial susceptibility test was carried out towards fifteen antimicrobial agents by using Vitek2 system with Antimicrobial susceptibility test cards. The results of antibiogram showed that the local isolates were possess highly resistance towards most antimicrobial agents under study. The high resistance wastoAmpicillin while the low resistance was to Imipenem.Two methods were used for detection of Extended Spectrum Beta Lactamases (ESBLs) production; first methods by using of Vitek2 system,thesecondmethods by using of polymerase chain reaction (PCR) technique to discoveryESBL encoding gene *bla*CTX-M-1-all gene. The results revealed the prevalence of ESβL encoding genes in local isolatesof*K. pneumonia(bla*CTX-M) 93.1% oftheisolates while 6.99% isolates were not harbored this gen.

Keywords*: KlebsiellaPneumoniae*, Resistant, β-lactam, ESβL,Cefepime,Imipenem, *bla*CTX-M, gene.

Introduction

Klebsiellapneumoniae is the most clinically pertinent Klebsiella species and is in charge of over 70% of human contaminations because of this genus[(1)]. In humans, *K. pneumonia*frequently colonizes the gastrointestinal tract, skin, and nasopharynx and is an imperative reason for genuine network beginning contaminations, for example, necrotizing pneumonia, pyogenic liver abscesses, and endogenous endophthalmitis[(2-3)].

The expanding predominanceof antibiotic obstruction and the absence of new antibiotic drug improvement has progressively decreased the treatment choices for bacterial diseases[(4-5)]. Extended-spectrum β-lactamases (ESBLs) keep on being a noteworthy issue in clinical setups the world over,resistant microorganismare developing worldwide as a danger to good result in the treatment of basic diseases in medical clinic settings. Amongst the wide exhibit of antibiotics, β-lactamis the most broadly utilized agents. Creation of β-lactamases is the greatest common cause of resistance to β-lactam antibiotics[(6-7)]. theproduction of β-Lactamase enzymes by the microorganisms hydrolyzedthe β-lactam ring amide bond. In latest years, there has been an growth in the rate and prevalence of ESBL producing infectious diseases. Two hindered or more types of extended spectrum β- lactamases (ESBLs) have been establish worldwide[(8)]. β-Lactam antibiotics are commonly used in the handling of infections caused by *K. pneumonia*. Medicinal isolates of this bacterium are disreputable for protecting a widespread range of β-lactamases and are obviously resistant to ampicillin and amoxicillin[(9-10)].

Material and Method

isolate collection

More than (392) clinical samples of *K. pneumoniae* isolates ware collected from some hospitals in Baghdad city from the period between October - December 2017. The isolates were taken from different clinical specimens including: sputum 12(8.39%) isolates, ear swabs 15(10.48%) isolates, burns 27(18.88%) isolates, blood 39(27.27%) isolates and urine 50(34.96%) isolates. Conventional methods (standardized biochemical methods) and Vitek2 system were used to identify the isolates.

Antibiotic susceptibility testing

The isolates were tested for 15 antimicrobial agents (Imipenem, Levofloxacin, Tazobactam, Ciprofloxacin, Nitrofurantoin, Clavulanic acid,Tobramycin, Gentamicin, sulbactam, Cefepime, Ceftazdime, Ceftriaxone, Sulfamethaxazole, Cefazolin and Ampicillin) by using of Vitek2 system with antimicrobial susceptibility test (AST 69) cards.Vitek2 system with the using of (AST69) cards was used for phenotypic detection of ESBLs production.

DNA extraction

Genomic DNA was extracted by (Geneaid GBB100) kit. DNA extraction was done according to the company instruction. Nanodrop framework was utilized to assess DNA fixation and immaculateness . Extraction DNA was stored at -20 ºC for PCR assay[11].

PCR Reaction Condition

MonoplexPCR was performed with GoTaq Green Master Mix. The primer which used to detect ESβL encoding gene was *blaCTX-M-1-all* (table 1). PCR mixture was set up for amplification of ESβL encoding gene *blaCTX-M-1-all* in a total volume of 25 µl contained within 12.5µl of Go Taq Green Master Mix, 4 µl of template DNA, 1 µl of the primer (10 picomole/ µl) and7.5 µl sterile nuclease free water.DNA amplification was completed with the accompanying warm cycling (initial denaturation 95 °C for 6 min, 35 cycles of amplification : denaturation 94 °C for 40 s, annealing 57 °C for 40 s, extension 72 °C for 50 s and final extension 72 °C for 6 min)[12].

Table 1: Sequences of the primer.

Primer	Sequences(5'…….. 3')	Product (bp)	Reference
CTX-M-1-all	F-ATGGTTAAAAAATCACTGCG R-TTACAAACCGTCGGTGACGAT	876	12

Finding and Discussion

Isolation and identification of *K. pneumoniae*

Out of 392 clinical samples only 143 samples were *K. pneumoniae*depending on colony morphology, Gram stain, biochemical test and Vitek2 system. Isolates ware collected from some hospitals in Baghdad city from the period between October - December 2017. A recent study by Ali and Sana'a (2019) found that Out of (140) clinical samples only (70) samples were *K. pneumonia* isolates[13]. The isolates were taken from different clinical specimens including: sputum 12(8.39%) isolates, ear swabs 15(10.48%) isolates, burns 27(18.88%) isolates, blood 39(27.27%) isolates and urine 50(34.96%) isolates (figure 1) show distribution of *K. pneumonia*e isolates in clinical specimens. The higher percentage was in urine while the lower percentage was in sputum.Study byIkeda*et al*.(14) establishmentof the 130 investigation cases, 68 and 62 cases showed the presence of *K. pneumoniae* in the sputum and urine, respectively[14].

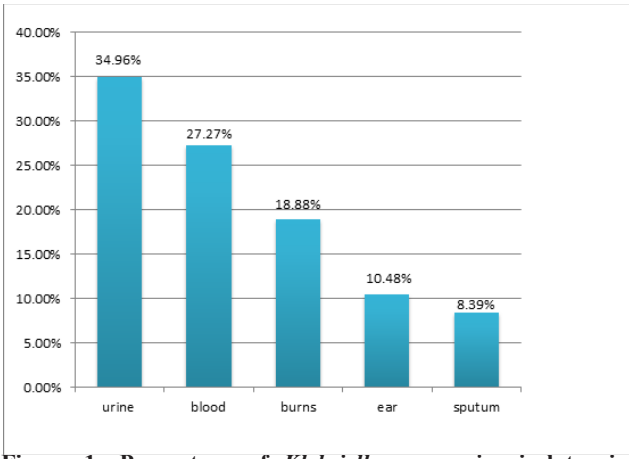

Figure 1: Percentage of *Klebsiellapneumonia*e isolates in clinical specimens

Antibiotic susceptibility

Antimicrobial susceptibility test was carried out towards 15 antimicrobial agents using by Vitek2 system with antimicrobial susceptibility test cardsnumber 69 (AST 69). The results showed that the local *K. pneumoniae* isolates were possess highly resistance towards most antimicrobial agents under

study. The patterns of resistance were as follows: 16.78% forImipenem, 24.47% for Levofloxacin, 26.57% forTazobactam andCiprofloxacin, 35.66% for Nitrofurantoin, 44.05% for Clavulanic acid, 58.04% for Tobramycin, 65.73% for Gentamicin, 81.11% for sulbactam, 84.61% for Cefepime,86.71% for Ceftazdime, CeftriaxoneandSulfamethaxazole, 88.11% forCefazolin, and 100% resistance for Ampicillin. All isolates were resistance toward ampicillin while the lower resistance percentage was toward imipenem as shown in figure 2.Study by Ali *et al*. (15) showed that the imipenem resistance was (58.97%) of *K.pneumoniae*isolates,the highest resistance rate was (100%)for ampicillin, ciprofloxacin was (10.26%) and levofloxacin reached to (7.69%)[15].

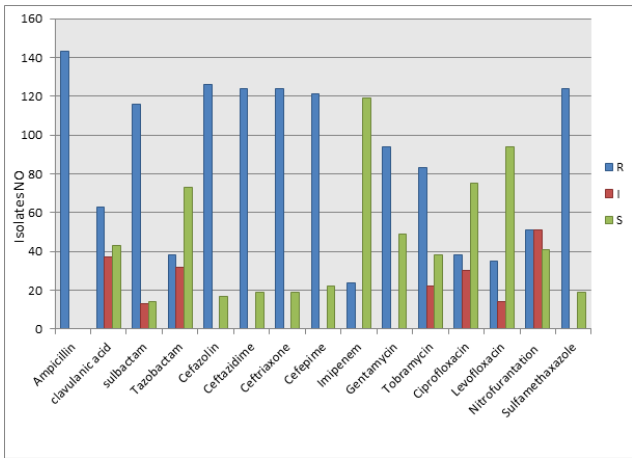

Figure 2: Antibioticssusceptibility for antimicrobial agents (R=Resistance, I= Intermediate and S= Sensitive).

Detection of ESβL encoding gene by amplification of *bla*CTX-M gene

Monoplex PCR technique was used for the detection of the*blaCTX-M* gene. The quick and strict diagnosis of the antibiotic resistance gene in the treatment is very important foravoiding the spread of infections. The present study aim was detectionof existence and prevalence of common ESβLs encoding genes(*blaCTX-M*)amongst143 clinical isolates of *K. pneumonia*. The results as in (figure 3)displayed the presence of *blaCTX-M* gene in 133 (93.1%) oftheisolates while 10(6.99%) isolates were not harbored this gen. while a study by Alyousef*et al.* (16)appear among the 97 *K. pneumoniae* isolates, 47.4% (*n* = 46) isolates demonstrated ESBL creation[16]. Expandingdevelopments of resistance to antibiotics due tocreation of β-lactamases. ESBL are the most across the board systems recognizable in *K. pneumoniae*.

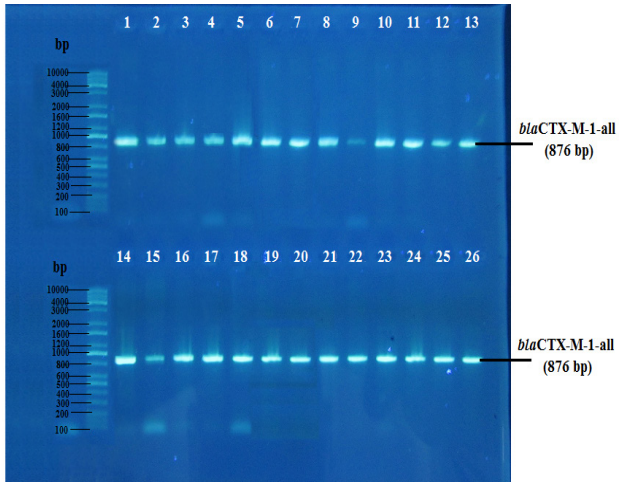

Figure 3: Gel electrophoresis (1% agarose,7v/cm² for 60 min) for PCR products *blaCTX-M-1-all* gene 876 bp lane M 100 bp DNA Ladder, lanes1-26: *K.pneumoniae* isolates. Detection was done on agarose gel (1.5%) at 5 V/cm for 1 hour, stained with RedSafe dye and visualized on a UV transiluminator documentation system.

Conclusion

From this research, it could be concluded that the higher percentage of *K. pneumoniae* isolates in urine and the lower percentage was in sputum,the local isolatesof*K. pneumoniae* were possess a highly resistance towards most antimicrobial agents under this study, all isolates were resistance toward ampicillin while there was low resistance percentage toward imipenem.high presence of *blaCTX-M* gene in theisolates.

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Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: All experimental protocols were approved under the Biology Department, College of Science, Mustansiriyah University, Iraq and all experiments were carried out in accordance with approved guidelines.

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Bond-Strength of Porcelain to Nobel and Non-Nobel Casting Alloys

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Abstract

Purpose: This study was designed to evaluate the bond strength of porcelain to white gold (Pd-Ag) and Nickel-Chromium (Ni-Cr) alloys. **Material and Method**: Rectangular-shaped specimens (25×3×0.5) mm were cast with white gold and Ni-Cr alloys and used as adherent materials. The Pd-Ag bonding surfaces were abraded with an airborne-alumina particle of 100 µm while that of Ni-Cr abraded with 250 µm. Specimens were fabricated by applying an opaquer and two dentin layer up to 1.1 mm in thickness at different cycling temperature. Bond strength for each group (n=7) was measured and compared statistically (α=0.05). **Results:** There were significant differences in bond strength of porcelain to Nobel and non-Nobel experimental alloys.

Conclusion: Based on the study results, it can be stated that the type of metal to ceramic alloys of different element composition may affect the porcelain bond-strength.

Keywords: *Bond-strength; porcelain; nobel alloy; non-nobel alloy; white Pd-Ag; and Ni-Cr*

Introduction

Metal to ceramic restoration has been widely used in the clinic since the 1950s. It provides a natural aesthetic of veneering porcelain alongside the marginal fit and the durability of metal casting alloy.[1-3]

Metal-ceramic restoration may contain Nobel and non-Nobel alloys. Nobel white gold alloy made up of gold, silver, nickel or palladium and chromium plating. The color is whitish similar to that of platinum and silver. White gold is considered to be an alternative to yellow gold, silver or platinum. It characterized by superior castability, ductility, corrosion, and malleability. Also, it provides easiness in polishing with high biocompatibility affect, [4]. On the other hand, Nickel-Chromium (Ni-Cr) considered as one of the non-Nobel alloys. The major component of the Ni-Cr elements by weight is 90% of Ni-Cr (Ni: 70-80%; Cr: 12-20%), 3-6% molybdenum, 2-6% aluminum, 0.5% beryllium and 4-8% silicon and manganese.[5]. Generally, the composition of the base alloy plays a major role in influencing on their properties such as surface hardness, Yield strength, and modulus of elasticity, corrosion, color, low weight, elongation and biocompatibility,[2,6-8].

In metal ceramic restoration, the demands for aesthetic restoration were increased,[9,10]. So, an application of porcelain opaque by mixing with the distilled water was used to block or mask the color of metal coping. This is to improve the natural color expression and to reduce the exposure of metal in the cervical area which aesthetically required. Also, it provides strength to marginal fit and limits the fracture in contactor areas,[11-13]. Clinically, the application of ceramic restorations have been increased, and the use of tooth-similar color materials made is possible to stimulate the appearance of the fabrication of highly aesthetic restorations,[14,15].

The strong bonding between the metal coping and the veneering porcelain is one of the essential requirements of clinically successful metal-ceramic restorations,[16-17]. The metal-ceramic bonding could either be chemically.[18-24], mechanically.[25-28] or due to generated forces such as Van Der Waals[,29,30] or coefficient of linear thermal expansion (CTE) between the metal and porcelain[31-35]. Therefore, this study was designed to assess the bond strength of the white gold alloy to porcelain in comparison with that of many Ni-Cr alloys at different casting treatment.

Material and Method

Material

In this study, the white gold was used as a Nobel alloy (Pd-Ag), while, the Ni-Cr of the different foundation was the non-Nobel alloy. The composition of the study alloys was detailed in table (1)

Table 1: The composition of the study alloys

White Gold Alloy (Pd-Ag) (Degussa, Germany)	Super 11 Ni-Cr (Argen dental, USA)	Bego Ni-Cr (Bego, Germany)	Kera NH Ni-Cr (Kera, Germany)	EX-3 Ni-Cr (Noritake, Japan)
Pd: 53.8%	Ni: 61.2%	Ni: 61.4%	Ni: 58.48%	Ni: 62.8%
Ag: 36.3%	Cr: 25.89%	Cr: 22.9%	Cr: 26.85%	Cr: 19.1%
Au: 0.7%	Mo: 11.09%	Mo: 8.8%	Mo: 12.73%	Mo: 7.1%
Sn: 7%	Si: 1.59%	Nb: 3.9%	Si: 1.72%	Ga: 1.9%
Ga: 2%	Mn: < 1%	Fe: 2.5%	Others: <0.1%	
Others: <0.2%	Fe: >1 %	Mn: 0.4%		
	C: >1 %,	Ti: 0.1%		
	Al: >1%			

Method

Sample preparation

A rectangular wax specimen design of (25x 3x 0.5) mm length, width, and thickness in dimension was prepared for bond-strength test,[4]. The wax patterns were connected to the plastic sprue cones at the middle line which in turn sealed to the plastic ring base that mounted on it to be ready to be invested.

The wax patterns were invested in using plastic rings. The vibrator was used to prevent the air bubbles to be trapped within the phosphate-bonded investment material (Rebi dental, Turkey). The investment was mixed and prepared according to manufacture instruction, then left for one hour for the final set.

After plastic ring removal, the set investment cylinder with wax patterns was placed in the furnace for wax burnout at 900°C and then left for one hour inside the furnace. According to the manufacturer instructions, the white gold and Ni-Cr metal alloys then were melted to be directed into the muffle mould entrance using casting centrifugal machine. After casting, the muffle was left to cool at room temperature and then the investment material was removed from the metal sample specimens.

The Pd-Ag specimens were finished using the abrasive wheel and the aluminum oxide (AL_2O_3) airborne-particle abrasion of 100 µm particle sizes. This applied for 20 seconds at air-pressure of 3 bars. To increase the surface area and enhance the porcelain wetting on the surface, the distance was of 20 mm from the metal specimen. On the other hand, the Ni-Cr specimens were abraded using of 250 µm of AL_2O_3 followed by ultrasonic cleaning in distilled water for 10min.

The Oxidizing layer was created for Pd-Ag group specimens while the degassing layer was performed for Ni-Cr groups as seen in figure (1).

Application of ceramic material

The porcelain was applied in layers (Vita-Germany) after mixing with distilled water (Salsal Company, Albasra, Iraq). An opaque (Op2, Vita-Germany) and two layers of dentin material (3L 2.5, Vita-Germany) were mixed with distilled water and applied using ceramic furnace (Ivoclar, Germany), and following the timetable

scheduled in the table (2) and as seen in figure (2). According to[4,36] the final thickness of 1.1 mm of the restoration was achieved by finishing and then finally polished specimen.

Figure 1: Oxidizing layer was created for Pd-Ag group specimens and the degassing layer was performed for Ni-Cr groups

Table 2: Scheduled timetable for application of ceramic to metal alloy study groups

Alloy	**Al_2O_3 Air-borne particles**	**Porcelain phase**	**Starting temperature (°C)**	**Heat rate (°C/min)**	**Firing temperature (°C)**	**Holding time (min)**	**Vacuum start (°C)**	**Vacuum end (°C)**
Pd-Ag	100 µm (20 Sec)	Oxidation	600	-	600	5	-	-
		Opaque	500	45	890	1	500	890
		Dentin	500	45	860	1	500	860
Super11 Ni-Cr	250 µm (20 Sec)	Degassing	550	45	980	5	550	980
Bego Ni-Cr		Opaque	450	60	920	1	450	920
Kera NH Ni-Cr		Dentine	550	60	900	1	550	900
Ex-3 Ni-Cr								

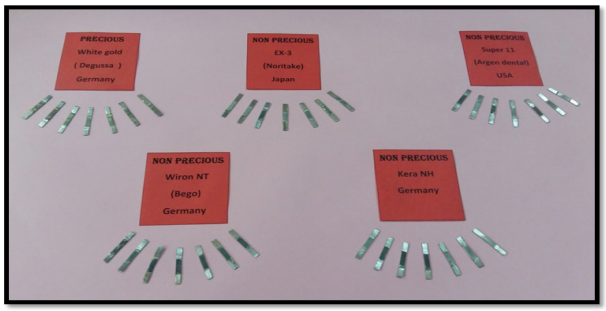

Figure 2: Firing of ceramic to metal alloys

Testing procedures

The specimens were tested using the computer-controlling universal testing machine (Laryee, China). According to ISO 9693 standards, the bond strength was calculated[,37,38].

Statistical Analysis

One-way analysis of variance (ANOVA) test was applied to analyze the study data using the SPSS (V-20),[39]. The statistical analysis revealed a significant difference among the means, and post hoc Games-Howell test was applied as seen in the table (3). Study tests were conducted at a confidence level of 95% (P<0.05).

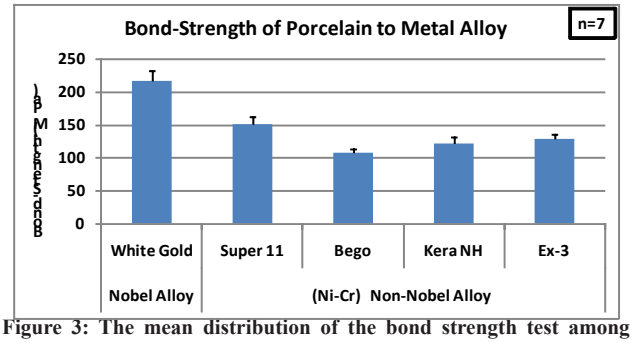

Figure 3: The mean distribution of the bond strength test among study groups

Table 3: Levene's test for study material

F	df1	df2	Sig.
2.795	4	30	.044

Results and Discussion

In this study and as seen in the table (4) and figure (3), the bond-strength of the porcelain to the white gold alloy was statistically significant than that of the Ni-Cr alloys (P<0.01). However, statistically non-significant differences were noticed between the Ni-Cr alloys of Kera NH and that of both Bego and EX-3 (P>0.05).

Table 4: One way ANOVA test for study groups

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White Gold Alloy	Super 11 Ni-Cr alloy	66.1429*	7.22571	.000	42.7414	89.5443
	Bego Ni-Cr alloy	109.4286*	6.18809	.000	87.5884	131.2687
	Kera NH Ni-Cr alloy	95.7143*	6.92575	.000	72.9687	118.4599
	Ex-3 Ni-Cr alloy	88.8571*	6.43333	.000	66.8627	110.8516
Super 11 Ni-Cr Alloy	Bego Ni-Cr alloy	43.2857*	4.66861	.000	27.3968	59.1747
	Kera NH Ni-Cr alloy	29.5714*	5.60976	.002	11.6388	47.5041
	Ex-3 Ni-Cr alloy	22.7143*	4.98910	.007	6.3236	39.1050
Bego Ni-Cr Alloy	Kera NH Ni-Cr alloy	-13.7143-	4.18939	.056	-27.7344-	.3058
	Ex-3 Ni-Cr alloy	-20.5714-*	3.31252	.000	-31.2648-	-9.8781-
Kera NH Ni-Cr Alloy	Ex-3 Ni-Cr alloy	-6.8571-	4.54382	.578	-21.5769-	7.8626

This study was designed to identify the bond strength between porcelain to white gold and Ni-Cr alloys at different heat treatment. Study specimens were prepared by using conventional laboratory techniques, equipment's, and materials used for construction crown and bridge. A universal testing machine was used to evaluate adhesion durability of metal bonded porcelain. The metal surface treated with porcelain to strength the bonding, this test determines the best bond strength from interface adhesion after surface treatment with porcelain to white gold and Ni-Cr alloys.

According to [29], Chemical connection is one the most important factor among the mechanisms of metal to porcelain bonding rather than the mechanical one,[29]. This may influence the metal-ceramic interface by the formation of the oxide layer which in turn affected by the compassion alloy. The chemical bonding of the oxide layer could result from the ionic or covalent bond between the oxides inside the porcelain and those oxides diffused from the metal surface. This may in agreement with,[11,12] as they conducted that effect of bonding by oxides.

Economically, the replacement of gold alloy by an alternative metal-ceramic alloy is on the rise up. A noble Pd-Ag alloy may offer a high biocompatibility alongside physical properties. This could due to less damage or deformity caused by casting. Yet, insufficient studies were introduced for the clinical application of Pd-Ag alloy in the dental restoration. The bond-strength may determine the alloy potential for bonding with porcelain by their oxidation behavior,[20,23,24]. According to the researchers, the nature of the metal-porcelain adherence may be indicated by the form of the oxides during the degassing cycle which in turn reflect the quality of the bond to porcelain,[20-22].

The resistance to bonding failure may be enhanced by the metal-ceramic interface of an appropriate oxide layer. The firing of porcelain may help in completely remove of the thin oxide layer. In addition, a weaker bond strength may notice as a result of low cohesive strength to thick oxide layer,[34,35].

Stable oxide layers in white gold alloy incorporating some elements such as tin may increase the bond strength of porcelain to such alloys.

According to,[16,17] the degassing of Ni-Cr alloy at a high firing temperature above 950 °C may produce excessively thick oxide layer. Regardless the manufacturer standards, alloys may require a different oxidation process instead of the traditional degassing procedure.

In addition, the alloy surface topography may improve the bond strength as well. Metals that treated by airborne-particles could improve the energy at the surface and increase the wettability of the porcelain. However, in the present study reported that the Ni-Cr alloy that sandblasted by 250 µm Al_2O_3 airborne-particle decreases in bond strength than that of Pd-Ag alloy of 100 µm. This could disagree with[,25] which stated that the highest bond strength could be achieved by using large particle to abrade surfaces.

According to[,4,31] the bond strength of flat interface of metal-ceramic can only measure the forces directly imposed on this boundary. As a result, the present study was performed in accordance with the ISO 9693 standards[,37]. The thickness of a metal plate and porcelain layer was clinically acceptable[,36].

Conclusions

Despite the limitation of this study, the results were concluded that the type of metal-ceramic alloys may affect the porcelain bond-strength. Ni-Cr alloy revealed a significantly decreased in bond-strength to porcelain in comparison with white gold alloy.

Conflict of Interest: No

Source of Funding : Self

Ethical Clearance: None

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Textile Wastewater Treatment by Application of Advance Oxidation (AOPs) Process and Recycling for Industrial Uses

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Abstract

In the present study, the treatment of industrial wastewater and recycling for industrial uses. Photocatalytic degradation of dyes like methylene Blue dye has been used as technique for treatment in a batch reactor under solar light in heterogeneous slurry utilizing various concentrations of commercial powder TiO_2as photocatalysts semiconductors. Also the parameters have been studied catalyst type, catalyst concentration, pH of dye solution and dye concentration at the beginning. Therefore the results show that the best dose of TiO_2 is 4.5 gm/l , and the optimum pH is 6. But the percentage removal of chemical oxygen demand (COD) of the dye solutions were for higher than that of the degradation of dye for the same conditions of catalyst.

While the water quality of treated wastewater depends on a greatextent of the quality for the industrial water supply, nature of thewastes added during use, and the degree of treatment the wastewater has received. Then wastewater quality data routinely measured andreported at the wastewater treatment plant are mostly for treatedeffluent disposal or discharge requirements in terms of gross pollution parameter.

Keywords: *Advance Oxidation Process ; Industrial Wastewater Treatment ; TiO_2 Semiconductor*

Introduction

Photocatalytic processes to degrade organicpollutantsinwaterbyutilizingcatalysthavebeenthe subject of research recently. Semiconductoroxidessuchas ZnO and TiO_2 have been recognized to be preferable materials for photocatalyticprocesses, dueto their high photosensitivity, non-toxicnature, lowcost and chemical stability, so thesemiconductororphotocatalysisis initiated bythesurfacetrappingofphoto-generatedelectrons(e^-) and holes(h^+), whichinducesinterfacialelectron-transfer reactions withadsorbedsubstrates. However, because of the wide band gap of ZnO and TiO_2[1,3]. A considerable problems in treatment plants caused by dyes presents in textile industry wastewaters since those compounds are complex to degrade by biological methods. Some of the effective chemical and physical methods state a high activity of color removals such as coagulation-flocculation, advanced oxidation and electrochemical methods [4,5]. All these methods have operational problems such as high sludge formation in chemical ways in addition to that more expensive[6,7]. Adsorption can be considered an unattractive method for degradation objective because of the cost of adsorbent and regeneration requirement. However, recent studies specified the possibility of using some natural or low-cost adsorbents for color removal such as wood, ash and soil[8,9].

In the present paper, the photocatalytic degradation of Methylene Bluedye in aquatic solutions using commercial powder TiO_2 as aphotocatalysts has been tested and recycle wastewater has been treated for agricultural uses. The major aims of the study were compared thechange of the following parameters withrespect to the degradation efficiency.Effect of catalyst concentration ;effect of pH ; effect of initial concentration of the dye; kinetic of reaction; and COD removal

Materials and Method

Materials

In present studycommercial TiO_2 powder were gained from Merck Co. (Germany Company), Methylene Blue dye was taken up from Merck (Germany Company)

and was applied without further purification. Distilled water was used fora preparation of different solutions. HCl (0.1 N) and NaOH (0.1 N) were used for change pH of solution.

Instruments

Photochemical degradation was carried out in specially designed insulation walled reaction containers with volume 800 ml under UV-light chamber equipped with 8 UV tubes each of18W (Philips) having a wavelength of 365 nm, so the spectra were taken with UV–vis spectrophotometer (ShimadzuUV-2101 PC) and chemical oxygen demand (COD) was analyzed withCOD meter (HANA HI9146). But constant stirring of the solution was covered by using magnetic stirrers. Then the temperature was kept constant during the reaction time by spreading the water in the photocatalytic reactor vessels.

Irradiation experiments

For 500 ml of the dye solution (25 ppm), photocatalyst was added with different concentration and a suspension was anexhibit to irradiation. While experiments were carried out under UV-light. The aqueous suspension was magnetically stirred over the experiment. At different time periods,an aliquot was taken out with the help of a syringe and then filtered through Millipore syringe filter of 0.45 µm[10,11].

Absorption Measurements

The absorption spectrum was recorded using double beam UV-2101(Shmadzospectrophotometer)compared with distilled water as a reference liquid and the percentage rate of degradation was noticed in terms of change in intensity at λ_{max} of the dyes during irradiation time[12,13]. While the degradation efficiency (%) has been calculated as:

$$\% \text{ Degradation} = [Abs_0 - Abs_t) / Abs_0] * 100$$

Where:-

% Degradation is a percentage of dye disappearance.

Abs_0 is initial absorption of dye (at time = 0 min.)

Abs_t is absorption of dye (at time = t min.)

Therefore the similar experiments haveused different catalyst concentration (2–5 gm/l), to select the bestconcentration of catalyst TiO_2at the same dye concentration of the solution (25 ppm).

Results and Discussion

Effect of Photocatalyst Concentration

The effect of catalystconcentration on the degradation of Methylene Blue(50 ppm) was investigated using commercialTiO_2 from (2 to 5 gm\L) keeping another parameters like pH , temperature , light intensity and dye concentrationconstant. The results in theFigure 1 showed that the degradation percentage increased with an increase in TiO_2 commercial concentration up to (4.5 gm\L for TiO_2). This observation can be explained in terms of availability of active sites on the catalyst surface and the permeation of solar light into the suspension. The total active surface area increases with increasing catalyst dosage. At the same time, due to an increase in the turbidity of the suspension, there is a decrease in UV-light permeation as a result of increased scattering effect and hence the photoactivated volume of suspension decreases. Furthermore, at high catalyst loading, it is difficult to maintain the suspension homogenous due to particles agglomeration, which decreases the number of active sites [14,15]. Also the results also shows the maximum photodegradation for commercial TiO_2 (4.5 gm/L) achieved after 150minute (see Figure 1).

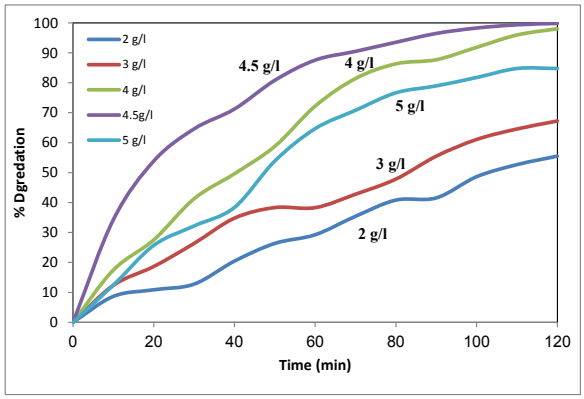

Figure 1 : Effect the catalyst dose of TiO_2 on degradation of Methylene Blue dye

Effect of pH on Solution Treatment

The wastewater is produced at different pH values, therefore, study of pH is very important on photodegradation of Methylene Bluedye. So experiments have been done at different values of pH varying from (4 to 12 for 20 mg/L) dye solution concentration and for the best of catalyst concentration (4.5 g/L)of TiO_2. But the figure 2 shows the percentage photodegradation

of against values of pH. It is clearly increasing in pH up to 11 for TiO_2because increasing in photodegradation activity [16]. Whilest the utilizing of TiO_2 as the catalyst is more appropriate at high pH values with the textile effluent. The interpretation of pH effects on the efficiency of the decolonization is a complex subject because many reactions can be occur to dye degradation such as "hydroxyl radical reaction, direct oxidation by the positive hole and direct reduction by the electron in the conducting band". Therefore the importance of each one depends upon the substrate nature and pH values[17,18].

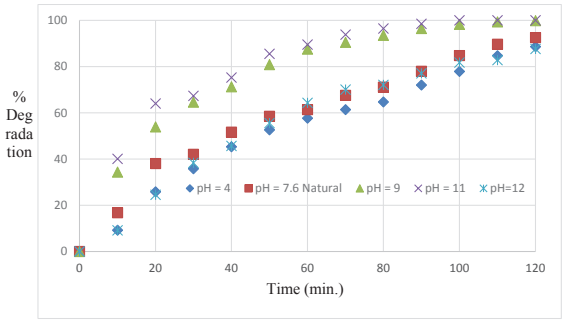

Figure 2: Effect the pH on degradation of Methylene Blue dye

Figure 2 : Effect the pH on degradation of Methylene Blue dye

Effect of Methylene Blue Dye Concentration

The photodegradation of Methylene Bluewas carried out by varying the initial concentration of the dye from (15 mg/L) in order to determine the effect of initial dye concentration on the best catalyst type and dose TiO_2. As the concentration of the dye was increased, the percentage of degradation decreased indicating for either to increase the catalyst concentration or time span for the complete removal. The figure3 depicts the time-dependent graphs of degradation of Methylene Blue at different concentrations of dye solutions(25–100 mg/L) . But in the case of dye solutions of (25 mg/L and 50 mg/L), 100% degradation occurred within 120 and 210minutes respectively. While in case of (75 and 100 mg/L) , the degradation with 240 minutes are 64% and 40%respectively,for the complete degradation of the dye and the percentage degradation further decreased on increasing the concentration of dye. Finally the reason for this behavior was the path length of the photons entering the solution decreases in highdye concentration that means the photochemical reaction is decreased but the number of photons absorbed by catalyst is high at low initial dye concentration the number of photon absorption by the catalyst in lower[19,20].

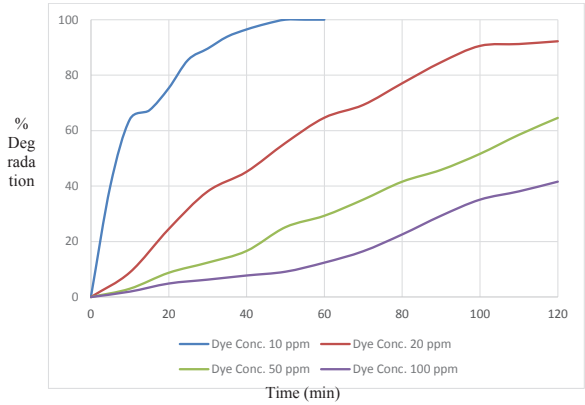

Figure 3 : Effect the dye concentration on degradation of Methylene Blue dye

Kinetic Study

Figure 4 shows the kinetic of the disappearance of Methylene Blue dye for an initial concentration of (50 mg/L). The results show that the photocatalytic degradation of Methylene Blue dye with commercial powder TiO_2concentration (1.3 gm/L)can be described by the first order kinetic model, $\ln (C_0/C) = kt$, where C_0 is the initial concentration and C is the concentration at any time t (y=0.0229x-0.2054). But the semi-logarithmic plots of the concentration data gave a straight line. Then the correlation constant for the fitted line was calculated to be $R^2 = 0.9739$ for commercial TiO_2[21,23].

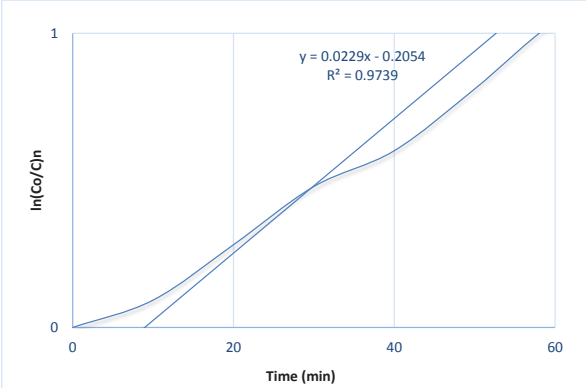

Figure 4 : Kinetic study of the disappearance of Methylene Blue dye

Conclusions

The photocatalytic degradation of aqueous solutions of Methylene Blue dye has been examined with the use of a UV-light-irradiated ZnO catalyst. It has been found that the process leads to decolorization and, eventually to complete mineralization of the dye solution. Also evolution of intermediates and final products on photocatalyst surface and the solution has been

monitored with a variety of techniques, which enable the identification of the reaction pathway, from adsorption of the dye molecule on the photocatalyst surface, to the formation of final products.Finally the photocatalytic degradation followed pseudo-first order kinetics.

Recommendation: We can develop this subject to covered agriculture uses or irrigation

Conflict of Interest: Non

Source of Funding : Self

Ethical Clearances: Nil

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Optimized photodegradation of reactive blue 19 on TiO_2 and TiO_2 suspensions, *Catal. Today* .2002. (76)(235–246).

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Spa gene Typing among *Staphylococcus Aureus* Isolates in Microbiology Laboratories of Scientific Research Center

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Abstract

This study aimed to *S.aureus* typing using gene encoding protein A (*spa* gene typing) in some clinical and environmental samples collected from microbiology laboratories at scientific research center

A total of 50 different environmental and clinical samples were collected from laboratories of ministry of science & technology (25 samples from environmental surfaces (benches, tables, sink,), 10 samples from mobile and 15 clinical samples. during the period from March / 2018 to May / 2018.

Biochemical and morphological characterization tests showed that thirty five isolates were identified as *Staphylococcus aureus* of it 20 isolates from environmental surfaces, 5 isolates from mobiles, 10 isolates from researcher's hands.

Antibiotic susceptibility tests of all isolates towards eight antibiotics were carried out and results showed that many isolates (25 %) were multi-resistant, DNA was extracted by wizard DNA extraction kit and it was amplified by specific primer of polymorphic X region of spa, which were determined by Ridom Staph Type software.

spa typing was carried out on all positive isolates. A total of 14 spa types was detected from 35 *S. aureus* isolates. t008 was the most common spa type (8; 29.6%), followed by t346 (4; 14.8%), t189 (2; 7.4%), t688 (2; 7.4%), t002 (2; 7.4%). All other spa types were less than 1% of *S. aureus* isolates.

The most common types of spa in our region were t008 (8; 29.6%), which indicate prevalence of community acquired *S.aureus*(CA-*S.aureus*) followed by t346 (4; 14.8%) from 27 spa types which were identified in this study.

In conclusion, we have shown that the laboratory environment could be a reservoir contributing to dissemination of virulent *S. aureus*, and The proposed surveillance protocol successfully allowed the detection of *S.aureus* contaminating important high-touch surfaces in a representative microbiology Lab. Frequently contaminated surfaces must be targeted for routine cleaning and disinfection.

***Key words** : spa gene,Staphylococcus aureus, PCR .*

Introduction

Staphylococcus aureus is the most common cause of human bacterial infections, it is a major global public health problem causing serious, often life threatening infections in the community and hospital setting that are becoming more difficult to manage with current antibiotics therapy regimens [(1)], *Staphylococcus aureus* is a commensal organism that colonizes nasal mucosa in 25-30% of the healthy human population [(2)], so it considered an opportunistic pathogenic bacterium due to its ability to take advantage of damaged cutaneous layers [(3)].

The pathology of a *S. aureus* infection is often dependent on which toxins are expressed and the virulence factors with which they are associated. It can cause infections ranging from mild skin irritation or a simple rash to severe illness including Toxic Shock Syndrome, abscesses, bone infections, and sepsis [(4)].

The primary cause of lower respiratory tract and surgical site infections and is the second leading cause of bacteremia, pneumonia, and cardiovascular infections.

The severity of infection is often dependent on which toxin genes are present, and the virulence factors associated with them[5].

S. aureus encodes many virulence factors including the surface Ig-binding protein A (*spa*) whose function is to capture IgG molecules in the inverted orientation and therefore prevent phagocytosis of the bacterial cells by the host immune system[6]**.** Genotypes known as "*spa*-types", based on highly variable Xr region sequences of the *spa*-gene, are frequently used to classify strains.

The purpose of this study was to examine the prevalence of *spa* gene of *S. aureus* in microbiology laboratories environment

Materials and Method

Sample Collection

Five microbiological laboratories were selected in ministry of science and technology, each sampling was selected based upon the most common areas touched by hands of staff working in the Lab, 25 samples from environmental surfaces (benches, tables, sink,), 10 samples from mobile and 15 clinical samples. during the period from(March / 2018 to May / 2018)were coded.

Samples Processing

Samples were collected using sterilized Swiffer pads as previously described. was it placed into a Whirl Pack™bag (Nasco, Fort Atkinson, WI), sealed, and added 50 ml peptone water ,then mixing well , after that was added 50 ml Baird Parker broth (2 times concentrations) with tellurite enrichment in 250 ml sterile screw cap jar and incubated for 18-24 hr at 37C. after incubation was inoculated onto Baird Parker agar and manitol salt agar and incubated 24-48 hr at 37C and examined for bacterial growth. Presumptive *S.aureus*(black colonies with clear halos on BPA) and then confirmed bydoing Gramstain, catalase test, coagulase test. Swabs were inoculated onto Baird Parker agar and manitol salt agar.

Antimicrobial Susceptibility Testing (AST)

S. aureus isolates were tested for susceptibility to benzylpenicillin, tetracycline, erythromycin, ciprofloxacin, trimethoprim-sulfamethoxazole, gentamicin, vancomycin, and nitrofurantoin using the Kirby-Bauer disk diffusion method on Mueller Hinton agar in accordance with the Clinical Laboratory Standards Institute standards and previously described methods[7].

Molecular Analysis

All isolates positive for *S.aureus* were selected for molecular testing.16SrRNA gene and nuc gene for the confirmation isolates, PCRwas carried out in a total volume of 25 µL[8]. For spa gene mixture to achieve 50 m LPCR products were run on a 1.5% agarose gel using electrophoresis, stained with gel red at 70 volts for 60 min and visualised under UV light using a gel documentation system .

Primers name	Sequences for (16SrRNA)	Product size(bp)
16SrRNA-R	5`-AGA CCC GGG AAC GTA TTC AC -3`	~800
16SrRNA-F	5`- GTG CCA GCA GCC GCG GTA A -3`	

Primers name	Sequences for (nuc) gene	Product size(bp)
Primer -R	**5′-GCGATTGATGGTGATAC-GGTT-3′**	**270**
Primer -F	**5′-AGCCAAGCCTTGAC-GAACTAAAGC-3**	

Determination of *spa* type was performed using published methods and primers . Based-Upon Repeat Pattern (BURP) analysis to identify *spa* cluster complexes (*spa*CC) was conducted using the RidomStaphType software using default parameters (version 2.2.1; Ridom, GmbH, Wurzburg, Germany).

The sequences of used primer are as follow:

Primers name	Sequences for (spa)	Product size(bp)
spa-1113f	**5'-ATC TGG TGG GGT AAC AACTG-3'**	1100
spa-1514r	5'-CGC TGC ACC TAA CGC TAA TG-3'	

Findings and Discussion

A total of 50 environmental and clinical samples

were collected from Five microbiological laboratories were selected in ministry of science and technology, The overall prevalence of *S. aureus* was 80% (20/25) for environmental samples, 50% (5/10) for mobile samples, 66.6% (10/15) for clinical samples respectively.

The most common places that were contaminated were areas touched showing up at 80% of total samples. This included bench,Sink, keyboards at the Laboratory. As environmental contamination increases, the prevalence of researcher hands carriage also increases [9]. This fact clarifies why these areas were the most contaminated[10]**.** The determinants that may have played a role in the areas that were most contaminated are hand hygiene among researcher [11], frequency of these areas being used, level of contamination among surfaces, and the amount of time and interaction that happens with environmental laboratory by researcher working in these areas[12].

There is a limited understanding of the dynamics and determinants of spread in this setting [13]with little research done in microbiology laboratory environments. Dynamics of *S.aureus* in hospitals have been sought, but the laboratory environment is still poorly understood[14].

Antibiotic susceptibility tests of all isolates towards eight antibiotics were carried out and results showed that many isolates (25 %) were multi-resistant. Overall (n=35), 94.2% (33/35) of isolates were resistant to penicillin, 65.7 % (23/35) were resistant to erythromycin, 60% (21/35) of isolates were resistant to meth prim, 48.5 % (17/35) were resistant to clindamycin , 40% (14/35) of isolates were resistant to tetracycline, 34.2% (12/35) of isolates were resistant to gentamicin, 22.8% (8/35) of isolates were resistant to chloramphenicol, 5.71% (2/35) of isolates were resistant to vancomycin. and 34.2.4% (12/35) were Multi-Drug Resistance (MDR) .

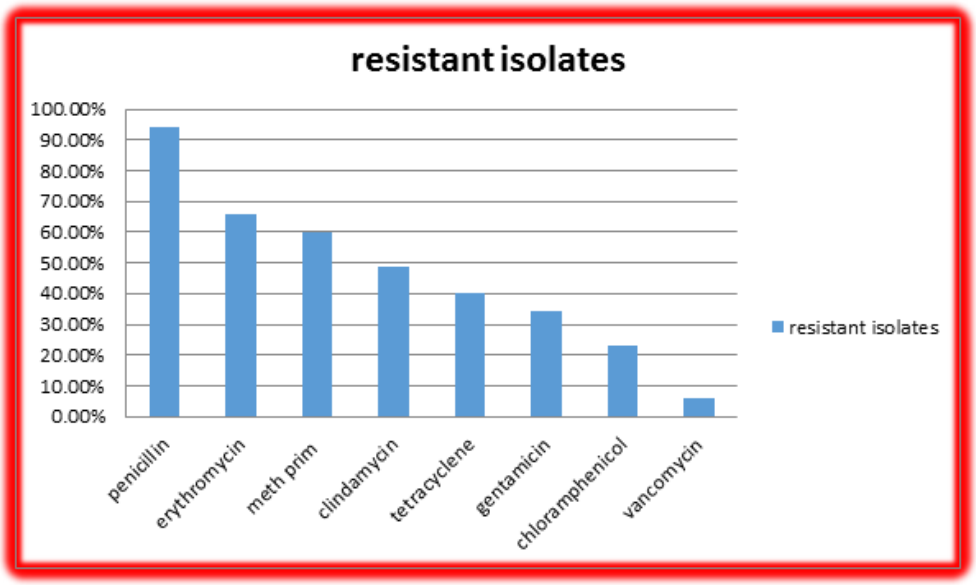

Figure-1- resistant isolates

Molecular characterization

Molecular characterization of all isolates were conducted by amplification of *spa* typing. The spa genewasamplifiedbyPCR forconfirmed *S. aureus* isolates . The control showed only one band of spa gene of 1100 bp (figure-4-)

14 spa types was detected from 35 *S. aureus* isolates. t008 was the most common spa type (8; 29.6%), followed by t346 (4; 14.8%), t189 (2; 7.4%), t688 (2; 7.4%), t002 (2; 7.4%). All other spa types were less than 1% of *S. aureus* isolates.

The most common types of spa in our region were t008 (8; 29.6%), which indicate prevalence of community acquired *S.aureus* (CA-*S.aureus*) followed by t346 (4; 14.8%) from 27 spa types which were identified in this study. (CA-*S.aureus*) appears to be more virulent than (HA-*S.aureus*). the clinical infections associated with it include severe skin and soft tissue infections and necrotizing pneumonia.

The concept of biosafety in laboratory practice is one that is of most important; and as such it must be given top priority at all times, and There is the need for biosafety to be placed in the first stage of issues in laboratory practice in over respective facilities [15]There must be a continuous concerted effort on the part of laboratories to ensure that their testing procedures are safe and in line with international best practices both for the safety of staff and patients and also to safeguard the immediate environment from potentially hazardous pathogens[16], The absence of appropriate biosafety policies and practices is one of the challenges facing researcher in laboratory

Table-1- *spa* gene typing

***Spa* typing item**	**No.of isolates**	**% of isolates with valid values**
t008	8	29.6%
t346	4	14.8%
t189	2	7.4%
t688	2	7.4%
t002	2	7.4%
t045	1	2.8%
t065	1	2.8%
t067	1	2.8%
t1149	1	2.8%
t1578	1	2.8%
t024	1	2.8%
t216	1	2.8%
t209	1	2.8%
t091	1	2.8%
t012	1	2.8%
t030	1	2.8%
t037	1	2.8%
t160	1	2.8%
t021	1	2.8%
t258	1	2.8%
t330	1	2.8%
t660	1	2.8%

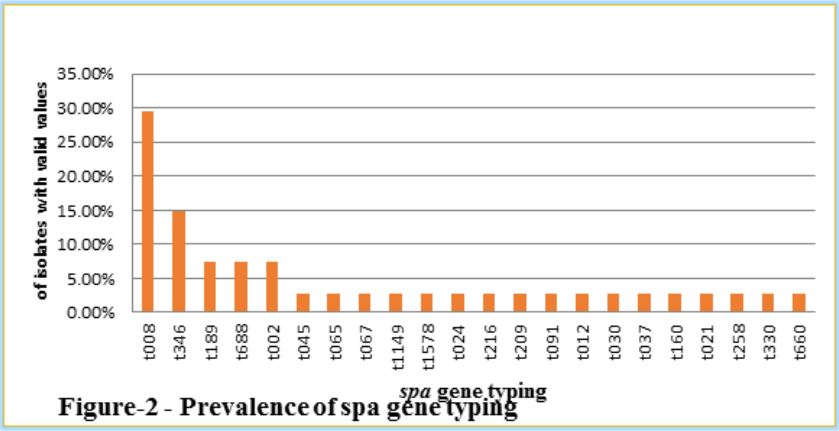

Figure-2 - Prevalence of spa gene typing

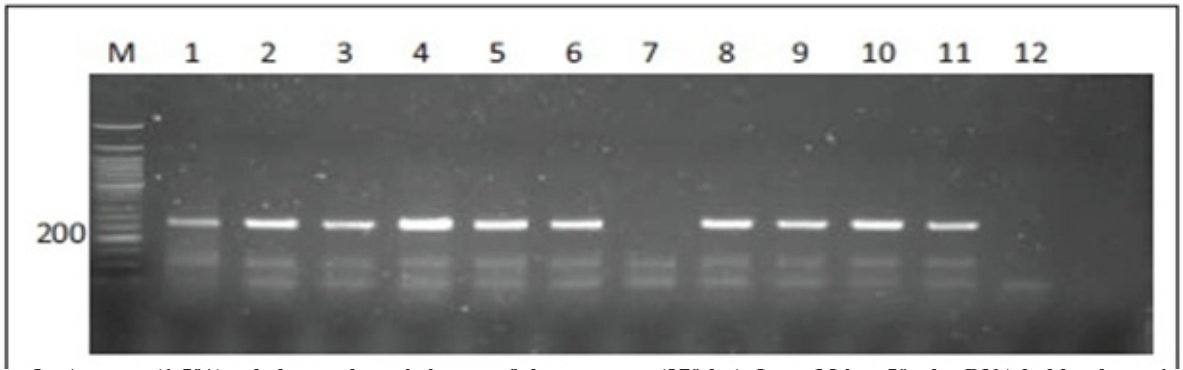

Figure – 3 - Agarose (1.5%) gel electrophoresis image of the nuc gene (270 bp). Lane M is a 50- bp DNA ladder, lanes 1 to 10 are test samples, lane 11 is a positive control and lane 12 is a negative control.

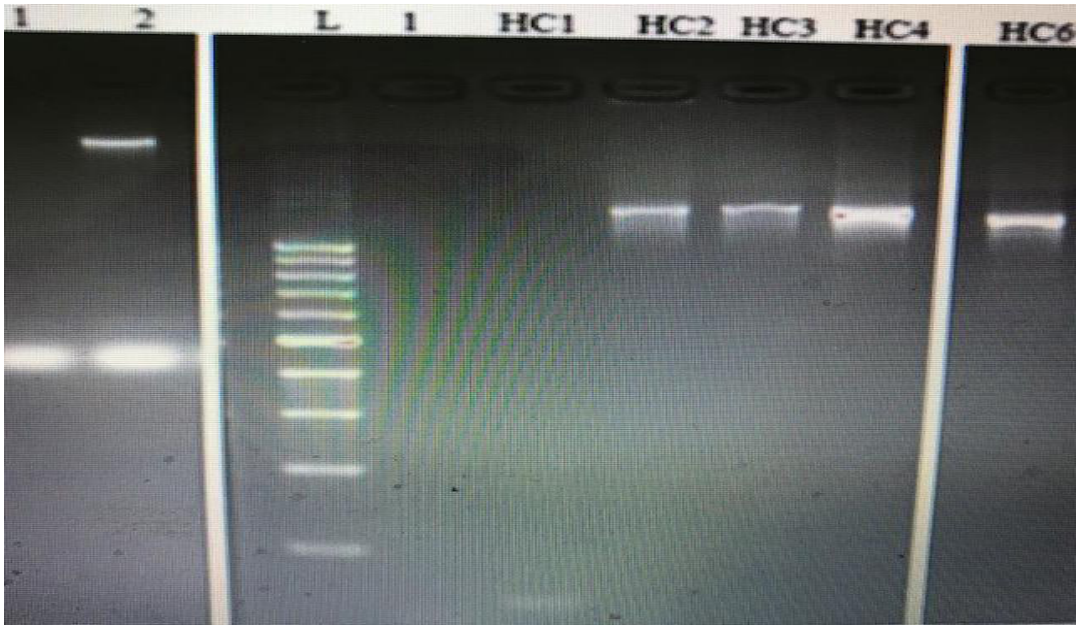

Figure – 4- Three percent agarose gel electrophoresis analysis of PCR amplificationproductsof spa gene of 1100bp, extracted from S. aureus PCR products for spa gene for positive MRSA samples. Lane L: DNA molecular size marker (100 bp ladder),

Recommendation and Conclusion

1- Rapid and accurate determination of the different spa gene prevalence of *Staphylococcus aureus* isolated microbiological laboratories are a great help in understanding the epidemiology of this bacteria in workplace area.

2- detection of bacterial contamination source in microbiology laboratories of all scientific research center using molecular typing.

3- comparison between microbiology and chemical Laboratory related bacterial contamination.

Conflict of Interest : None.

Source of Funding: Self funding **.**

Ethical Clearance: Scientific research projects of Ministry of Science of Technology.

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Nutritional Knowledge and Practices amongpregnant Women attending Primary Health Care Center in Baghdad City

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Abstract

Objective: To estimate the grade of knowledge and practice of nutrition among women of pregnancy. **Method:-**A cross-sectional study carried out in at al- Kadmayha& Bab-almoadham primary health care. The sample was selected by (non probability convenient sampling) and sample size was (100). Data was collected done by interview to take out demographic information. **Results:-** The result display that the multitude of cases were (45%) through age group (20-29) years. As for education (3%) were illiterate As for occupation (61%) were workers and about (39%) housewife. and this research explore that (67%) of gravid female had kindly knowing and about (56%) of gravid women had good practice on feeding pending pregnancy. Conclusions: This project displays the factors that found significant combination with nutrition knowledge.Consist of standard of awareness and nourishment acquaintance through conception and significant convenient relevance between the age group and nutrition habit during pregnancy.

Key words*: knowledge, practice, factors, nutrition, pregnancy, Baghdad*

Introduction

Nutrition during preconception as long as throughout pregnancy has a central influence on the impactof pregnancy [1]. Balanced and adequate maternal assimilation of macro nutrition and micronutrients guaranty not only the health situation of the pregnant mum, the luxury of the foetus and the newborn but future soundness status of herself (safeguard of life) and her three descent (protection of progeny) [2, 3] .Besides, pauper and extravagant alimentation of gravid women may leading to maternal harm and death[4, 5]. Nutritional imponderables causes fail of the embryo to extent its full development prospect recognized as embryonic growth limitation, which is combined with child together little and long duration complications, mortality and mortality[6, 7].

On the inversion women who are suffering from malnutrition previous and throughout pregnancy are probable to experiment inverse pregnancy consequence. Before pregnancy the woman needs nutrients for growth and maintenance of her body. Good nutrition keeps her healthy. During pregnancy additional requirement for all nutrients occurs to enable the fetus to grow normally in the uterus [8].

Healthy eating behavior in pregnancy support to inhibit pregnancy complications, aids reform from childbirth, efficiently tolerate breastfeeding and also prevents the manifestation of diseases in maturity [9, 10]. The incidence of dietary supplement impairment as an outcome of dietary attitude and modality in pregnancy is major than during pregnancy than at any other juncture of the life period, It was shown that, nutrition knowing was oracular of alteration in dietary habits and health exhortation motivate expecting women to amelioration their food assimilation [11].

It was usually reasonable that the fetus was feed adequately at the expenditure of maternal storage and needs; however, it is becoming clear that this may not always be the case, and that fetal expansion can be less than optimal if certain nutrients are not obtainable during intrauterine life [12]. Therefore the advancement of female validity and other preventive health auspices practice should onset before birth, during intrauterine life and expand by means of various phases of their lives in order to incur their general as well as their reproductive health [13].

Method

Design of the Study:A cross sectional study design (convenient sampling) for pregnant women was conducted starting from (1st September 2018 to 1st of March 2019).

Setting of the Study: The study is conducted atal-Kadmayha& Bab-almoadham PHC centers.

The sample of the study: A convenient sampling, purposive sample of 100 female.The fact was collected by direct interview using special questionnaire to acquired socio-demographic information. (Age, education, occupation……..ect

Scales and scoring:

The items have rated and scored accord to the following patterns:

1. Knowledge scale: three points likert scales are applied for rating the knowing of study sample items as score of three for (correct answer), 2 for (don't know) and score 1 for (false answer).
2. Practice scales: three points are used for rating items as score of three for (always), 2 for (sometimes) and score one for (never).
3. Determination of a grades of the samples knowledge was Divide into three faces (poor, fair , and good)
4. $$\text{Rating Range of score} = \frac{\text{Max-Min}}{3} = \frac{3-1}{} = 0.66$$
   - Poor knowledge score : 1-1.66 (<55%)
   - Fair knowledge score : 1-67-2.33 (56-78%)
   - Good knowledge score :2.34-3 (>78%).
   - Determination of a grades of the samples practice was be included three grades (poor, fair , and good):-
   - Poor practice e score : 1-1.66 (<55%)
   - Fair practice score : 1-67-2.33 (56-78%)
   - Good practice score :2.34-3 (>78%).

Data analysis by SPSS version 18, x^2 test was appliedfor significance of p value of <0.05 was considered significant.

Findings

Table 1:- Distribution of sample according to age, education level occupation, Receiving information and exporter of input and nutritional habits.

Variables		No 100.	%
Age	<20	17	17.0
	20-29	45	45.0
	30-39	38	38.0
Education level	Illiterate	3	3.0
	Read and write	4	4.0
	Primary	15	15.0
	Intermediate	11	11.0
	Secondary	18	18.0
	College and above	49	49.0
Occupation	workers	61	61.0
	housewife	39	39.0
Receiving information	Yes	94	94.0
	no	6	6.0
Sources of information	Health staff	47	47.0
	Pregnant women	11	11.0
	Friends	15	15.0
	Family and relative	14	14.0
	Media	13	13.0
	Total	100	100.0
***Nutritional habits**	Using salt to cook meal	22	22
	Eat fresh fruit	25	25
	Taking dark tea	11	11
	Snack food	10	10
	Drinking milk	55	55
	Eating milk product	56	56
	Eating meat	44	44
	Iron supply	77	77
	Folic acid supply	78	78

***nutritional habit collect answer range from all pregnant women**

Table (1) shows that of the (100) pregnant women there were (45%) in age group (20-29) years. As for education (3%) were illiterate while (49%) had higher education. As for occupation (61%) were workers and about (39%) housewife . Receiving information on nutrition about (94%) were yes, as for Sources of

information (47%) were health staff, (78%) folic acid supply (10%) was drinking dark tea.

Table 2:- summary of nutritional knowledge and practice score

Knowledge score	**Frequency**	**percent**
good	67	67.0
poor	33	33.0
Total	100	100.0
Practice score	Frequency	percent
good	56	56.0
poor	44	44.0
Total	100	100.0

Table (2) shows that (67%) of pregnant female have good knowledge regarding appropriate nutrition according pregnancy. While (56%) of pregnant women have good practice regarding appropriate nutrition during pregnancy..

Table(3): relation between Knowing Level and age of pregnant women, education and occupation

Variables		**KNOWLEDGE SCORE**						
	good		**poor**		**Total**			
	No	%	No	%	No	%	p-value	
Age	<20	10	14.9	7	21.2	17	17.0	
	20-29	35	52.2	10	30.3	45	45.0	**0.116**
	30-39	22	32.8	16	48.5	38	38.0	
	Total	67	100.0	33	100.0	100	100.0	
Education	Illiterate	1	1.5	2	6.1	3	3.0	
	Read and write	3	4.5	1	3.0	4	4.0	
	Primary	9	13.4	6	18.2	15	15.0	
	intermediate	9	13.4	2	6.1	11	11.0	**0.0016**
	Secondary	12	17.9	6	18.2	18	18.0	
	college	33	49.3	16	48.5	49	49.0	
Occupation	workers	41	61.2	20	60.6	61	61.0	0.955
	Housewife's	26	38.8	13	39.4	39	39.0	

Table (3) shows that good knowledge of pregnant women who were 20-29 years old is greater than those who were lower than 20 years and more than 29 years old. There. As for education that poor knowing of pregnant women whose education was illiteracy, There was statistically significant relationship between them with p-value <0.00. As for occupation shows that good knowledge who were workers was greater than those who were their housewife's.

Table(4): Relation between Knowledge Level and Receiving information

Variables		**KNO WLEGE SCOR**						
	good		**poor**		**Total**			
	No	%	No	%	No	%		
Receiving information	Yes	66	98.5	28	84.8	94	94.0	0.007
	No	1	1.5	5	15.2	6	6.0	
Sources of information	Health staff	32	47.8	15	45.5	47	47.0	0.745
	Pregnant women	7	10.4	4	12.1	11	11.0	
	friends	10	14.9	5	15.2	15	15.0	
	Family and relative	11	16.4	3	9.1	14	14.0	
	media	7	10.4	6	18.2	13	13.0	

Table (4) presentation that gravid women whose receiving information on nutrition had good knowledge about (98.5%).High statistically significant relevance between them with p-value <0.00.As for sources of information that good knowledge of gravid women who were health staff.

Table (5): Relationship between practice Level and age of pregnant women, education and occupation

		PRACTICE SCORE						
	good		**poor**		**Total**			
	No	%	No	%	No	%	p.v	
Age	<20	8	14.3	9	20.5	17	17.0	**0.063**
	20-29	31	55.4	14	31.8	45	45.0	
	30-39	17	30.4	21	47.7	38	38.0	
	Total	56	100	44	100	100	100.0	
Education level	illiterate	3	5.4	0	.0	3	3.0	**0.021**
	Read and write	2	3.6	2	4.5	4	4.0	
	Primary	8	14.3	7	15.9	15	15.0	
	Intermediate	6	10.7	5	11.4	11	11.0	
	Secondary	9	16.1	9	20.5	18	18.0	
	college	28	50.0	21	47.7	49	49.0	
Occupation	Workers	37	66.1	24	54.5	61	61.0	**0.241**
	Housewife's	19	33.9	20	45.5	39	39.0	

Table (5) shows that good practice of pregnant women who were 20-29 years old is great than those who were less than 20 years and more than 29 years old. As for education that good practice of pregnant women whose education was higher, As for occupation shows that good practice who were workers was higher than those who were their housewife's.

Table(6): Relations ofpractice Level

		PRACTICE SCORE						
	good		**poor**		**Total**			
	No	**%**	**No**	**%**	**No**	**%**		**p.v**
Receiving information	yes	54	96.4	40	90.9	94	94.0	**0249**
	no	2	3.6	4	9.1	6	6.0	
Sources of information	Health staff	29	51.8	18	40.9	47	47.0	**0.383**
	Pregnant women	6	10.7	5	11.4	11	11.0	
	friends	5	8.9	10	22.7	15	15.0	
	family	9	16.1	5	11.4	14	14.0	
	media	7	12.5	6	13.6	13	13.0	

Table (6) display that pregnant women whose receiving information on nutrition had good practice about (96.4%).As for sources of information that good practice of pregnant female who were health staff.

Discussion

The period of pregnancy is remarkable by enormous physiological variation that demands healthy dietary lifestyle option in arrangement to enhance the physical and psychological development of the unborn child [4]. The nutritional state of pregnant woman remains critical in determining healthy pregnancy outcomes. In this study, most of the pregnant women belongs to the age 20-29 years the finding of the current study is agreement with findings reported in Ethiopia [14], and in Nigeria [15], this may be because the extreme ages of reproductive years are well know about nutrition during pregnancy.

And almost about (49%) of them were collage education the finding of the attend study is agreement with findings reported in Nigeria [15],found a highest knowledge score were obtained by women of higher education. Health staff in this study was the source of information for 47% of the pregnant women. The outcome of this research was more than the study proceed in Gambia [16], that showed the provenance of acquaintance for the study participants were 35% being informed on nutrition and feed by their antenatal nutrition care providers .

Although the outcome of this research was additional than the study conducted in Gambia much effort was consequence needed to get better nutrition teaching to adequately supply the necessary information which is crucial to prevent the Intergenerational effect of malnutrition. The result of this study also present that the ratio of mothers who have appropriate knowledge on maternal nutrition during pregnancy was found to be 67%.

As a consequence of this study is similar with the study proceed in Ethiopia [14], in which great than semi of the women in the study had the basic and essential knowledge concerning prominence of nutrition during pregnancy . This result also similar the study completed in Malaysia[17], 65.7 % and Swaziland [18], 67% of mothers had compatible knowledge across maternal nutrition .

Whereas the finding of this research is more than the study finished in Ethiopia[19], 52.5% of mums had

compatible knowledge related maternal nutrition . A this discrepancy might be regarding to socioeconomic factors and cultural difference of the study participants. The peak level of nutrition knowledge demonstrated in our study maybe due to high educational level of the participants .

The finding of this study was also reveal that the proportion of gravid women with good pregnancy specific nutrition practice was set up to be 56%. Similarly the study conducted in Swaziland [18], reported that 51% pregnant women had good practice regarding nourishment during gravid . On the other hand a higher proportion of gravid women with good nutrition workout were notify from another study conducted in Malaysia 74% and Pakistan 65.5% [17, 20].

The potential reasons for these variation might be regarding to the diversity in socioeconomic status, cultural believes, access to nutrition and health services among the study participants. This study shows the age was not important predictor of knowledge of graved female in the research area. The results of the discuss are not comparable with the results from Ghana [21], where women aged 25 to 35 years had the elevated ratio of dietary knowledge, compared with those younger than 25 and older .The probable reason for observed discrepancy may be the difference in socio-cultural of the two-study population with respect to food and exposure to nutrition information during pregnancy.

Educational level, Nutrition information has been specified during pregnancy and the importance of women's knowledge of nutria during pregnancy among the study is similar to the one in Malaysia [22], demonstrated that women with preferable nutritional knowledge are significantly higher in educational level, nutritional formation .

The finding of the research stated that individuals with a higher educational standard had best nutrition knowledge in study may be explained by more access to internet, books and magazines as source of information in work area [22]. This study shows that there was statistically significant company between dietary practices of mothers and age the finding of the present discuss is disagreement with findings reported in Ethiopia [23],found that age had no combination with dietary practices of mothers. This could be explained by different in time and place of the study.

Conclusions

The finding of the study fixed that (67%) of pregnant women had good knowledge on nutrition during pregnancy. The results of the study particular that (56%) of pregnant women had good practice on nurture during pregnancy.

There was a correlation between you and a statistical indication between the level of education, information and nutrition there was significant positive relation between the age and nutrition practice during pregnancy.

Source of Funding: Self funding.

Ethical Clearance: Non

Conflict of Interest : None.

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Levels of Calcitonin and Procalcitonin in Different Types of Thyroid Cancer in Kirkuk City-Iraq

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Abstract

Thyroid cancer is the most common endocrine malignancy, accounting for 1.9% of all new malignant tumors. Thyroid cancer begins in the follicular cell of the thyroid gland. The aim of the study was to estimate the level of procalcitonin and thyroid tests in patients with different types of thyroid cancer. The number of thyroid cancer women under study were 40 women whose ages were between 25-75 years old were included in this cross-sectional study which carried out in Kirkuk city from the period of September 2016 to March 2017. These patients admitted to Kirkuk oncology center. The control group who were matched to the thyroid cancer patients studied, included 30 heathy individuals (blood donors). Blood was collected from patients and control for estimation of serum total TSH, T3, T4, FT3, FT4, and procalcitonin using immunefluorescent technique. The study showed that that the highest rate of increased PCT level was recorded among thyroid cancer patients comparing with the control group (25% *v.s.* 10%), the highest mean level of PCT was noticed among medullary thyroid cancer (8.22 ± 3.16 ng/ml) comparing with follicular and papillary thyroid cancer. The highest mean levels of TSH, T3 and T4 were noted among thyroid cancer patients (16.3± 4.22, 2.01 ± 0.55 and 144.1 ± 45.6) respectively comparing with the control group which showed the least means of TSH, T3 and T4 and with highly significant difference between the two groups. The study showed that the lowest mean of TSH was noted among MTC patients (0.88±0.61) comparing with FTC and PTC patients and the high means of FT3 and FT4 were noted among MTC patients (5.89±0.31and 19.25±2.48) respectively. Table 5 demonstrates that the lowest mean levels of FT3 noted among thyroid cancer patients comparing with the controls (2.99±10.91 *v.s.* 15.91±0.43). It was concluded that there was a highly significant relation procalcitonin and thyroid cancer specially medullary type.

Keyword: *Thyroid cancer, MTC, Procalcitonin, Thyroid.*

Introduction

Thyroid cancer is the most common endocrine malignancy, accounting for 1.9% of all new malignant tumors. Thyroid cancer begins in the follicular cell of the thyroid gland [(1)]. There are 2 types of cells located within the thyroid parenchyma: the follicular cells and the supporting cells (also called the *C cells*) [(2)]. Cancers derived from follicular cells are generally differentiated thyroid carcinomas (DTC). Although these cancers are not usually aggressive, they can eventually mutate into more aggressive variants. They have been traditionally classified as well differentiated thyroid carcinoma, including papillary thyroid carcinoma (80%) and follicular thyroid carcinoma (PTC and FTC, respectively) (10-15%)[(3)]. Medullary thyroid carcinoma (MTC) accounts for up to 5% of thyroid cancers, four in five cases occur as a sporadic and the remaining 20% are part of familial disorders [(4)]. The detection of MTC still represents a challenge and not infrequently MTC is only diagnosed histologically after surgery done for nodular goiter. This is due to several reasons[(1)]. In particular, several technical pitfalls can affect the measurement of serum calcitonin (CT) which represents the main presurgical and postoperative serum marker of MTC; no fixed CT thresholds to diagnose and exclude MTC are available and non-specific increase of CT in several non-thyroidal conditions may exist; MTC with undetectable CT is rarely reported [(5)]. Thus, further markers are

advocated to better manage MTC cases before and after their initial treatment. Traditionally, MTC patients are treated by thyroidectomy and central (with or without lateral) neck-dissection, and after surgery CT is the pivotal tumor marker[7,8]. However, several limits affect CT accuracy. High CT might be observed in C-cell hyperplasia, non-thyroidal neoplasia (neuroendocrine tumors, leukemias, systemic mastocytosis, small cell lung, breast and pancreatic cancer) renal failure, endocrine disfunctions [9]. The aim of the study was to estimate the level of procalcitonin and thyroid tests in patients with different types of thyroid cancer

Material and Method

The number of thyroid cancer women under study were 40 women whose ages were between 25-75 years old were included in this cross-sectional study which carried out in Kirkuk city from the period of September 2016 to March 2017. These patients admitted to Kirkuk oncology center. The control group who were matched to the thyroid cancer patients studied, included 30 heathy individuals (blood donors). 5 ml of blood was collected and placed in plane tubes, left for 30 minutes at 37 °C for clotting and centrifuged at 3000 rpm for 15 minutes, the obtained sera was aspirated using automatic micropipette and transferred to Eppendorf tubes and stored in deep freeze at -20°C for for estimation of serum total TSH, T3, T4, FT3, FT4, and procalcitonin using immunoflurescent technique (Minividas Immunofluorescent, France).

2.2. Statistical analysis: Computerized statistically analysis was performed using Mintab ver 18.0 statistic program. Comparison was carried out using Chi-square (X^2) for dtermination of The *P.* value.

Findings

Table 1 showed that the highest rate of increased PCT level was recorded among thyroid cancer patients comparing with the control group (25% *v.s.* 10%) with highly significant difference between the two groups regarding PCT level. In relation of PCT level with the type of thyroid cancer the study showed that the highest mean level of PCT was noticed among medullary thyroid cancer (8.22 ± 3.16 ng/ml) comparing with follicular and papillary thyroid cancer with significant differences among them(Table 2).

Table 1: Relation of PCT level with thyroid cancer patients and the control group.

Procalcitonin (PCT)* (ng/ml)	Thyroid cancer		Control	
	No.	%	No.	%
increased	10	25	3	10
Normal	30	75	25	90
Total	40	100	30	100

P. value: 0.031

Table 2: Differences of PCT levels among types of thyroid cancer

PCT ng/ml	Thyroid cancer (n:52)		
	Follicular (n:5)	Medullary (n:5)	Papillary (n:30)
Mean	0.49	8.22	0.93
S.D	0.12	3.16	0.45

P. value: 0.008

In Table 3, the highest mean levels of TSH, T3 and T4 were noted among thyroid cancer patients (16.3± 4.22, 2.01 ± 0.55 and 144.1 ± 45.6) respectively comparing with the control group which showed the least means of TSH, T3 and T4 and with highly significant difference between the two groups. The study showed that the lowest mean of TSH was noted among MTC patients (0.88±0.61) comparing with FTC and PTC patients and the high means of FT3 and FT4 were noted among MTC patients (5.89±0.31and 19.25±2.48) respectively, Table 4.

Table 3: Relation of thyroid hormones mean levels with thyroid cancer and the control group.

Parameters		**Thyroid cancer (n:40)**	**Control (n:30)**	***P. value***
TSH µU/ml	Mean	16.3	1.22	0.009
	S.D	4.22	0.81	
T3 pg/mL	Mean	2.01	2.477	0.035
	S.D	0.55	0.41	
T4	Mean	144.1	108.4	0.09
	S.D	45.6	33.9	

Table 4: Relation of thyroid hormones with cancer type

Parameters (mean ± SD)	**Thyroid cancer (n:40)**			***P. value***
	Follicular (n:5)	**Medullary (n:5)**	**Papillary (n:20)**	
TSH	13.5±6.9	0.88±0.61	18.3±8.2	0.74
T3	2.1±0.8	2.88±0.81	3.88±0.91	0.941
T4	83.5±6.1	144±12.1	128.3±51.7	0.48
FT3	3.44±1.55	5.89±0.31	2.91±1.22	0.37
FT4	7.22±3.18	20.6±2.34	113.2±4.9	0.22

Table 5 demonstrates that the lowest mean levels of FT3 noted among thyroid cancer patients comparing with the controls (2.99±10.91 *v.s.* 15.91±0.43) with highly significant relation, while FT4 was slightly decreased in thyroid cancer patients comparing with the controls (1.83±6.99 *v.s.* 2.61) with no significant difference between the two groups regarding FT4 levels.

Table 5: Relation of FT3 and FT4 mean levels with thyroid cancer and the control group.

Parameters		Thyroid cancer (n:40)	Control (n:30)	*P. value*
FT3	Mean	2.99	15.91	0.0002
	S.D	0.91	0.43	
FT4	Mean	13.83	16.33	0.26
	S.D	6.99	2.61	

Discussion

Procalcitonin is determined by several analytical tests with different immunore active isoforms and fragments which leads to poor comparability of results [7,8]. In addition, even if PCT stimulation may improve its reliability, there is high variability of results from different articles in different populations. For all these reasons, the management of MTC in clinical practice may hide some pitfalls. In example, some MTC patients show a persistent biochemical disease [7,8]. The studies of D'herbomez *et al* [10] emphasize the need of PCT assessment every time the results of CT measurement are unclear. The authors noted the interference between CT and PCT detection in the case of the use of a sensitive immunometric assay. Abilities of procalcitonin and calcitonin to distinguish MTC from other conditions, revealed comparable utility [11]. In previous studies better performance of PCT in comparison with CT have been recorded. Specifically, when the same antibodies were used, all of the commercial PCT assays yielded similar results[12]. One study noticed that the evaluation of PCT as a marker for the initial preoperative detection of MTC[13]. Kaczka *et al* [14] showed that PCT levels were higher in all active MTC patients mean PCT 3.5 ng/ml with active carcinoma which was markedly elevated levels and significantly higher than in the control group.

Antonelli *et al* [15] demonstrated that TSH, and other thyroid hormones levels were decreased among thyroid cancer patients specially in MTC type. This study supports that serum TSH concentration at presentation is an independent predictor of thyroid malignancy. We demonstrated that the risk of malignancy increased in parallel with serum TSH concentration at presentation which is in agreement with previously published data by Boelaert *et al* [16] Although our study included fewer patients when compared with Boelaert *et al* (1500 patients). Fiore *et al* [17] reported in their study that the serum TSH was significantly higher in PTC than in patients with benign nodular thyroid disease, whereas serum concentrations of both free T3 and free T4 were not significantly different, this observation is relevant on clinical grounds to define the risk of PTC in patients with nodular thyroid disease. In this regard, Rago *et al* [18] evaluated possible clinical and biochemical criteria useful to predict the likelihood of thyroid malignancy in patients with thyroid nodules. It stands to reason that if the levels of TSH are elevated in patients with thyroid cancer, it is possible that elevated levels of thyroxine (T4) and triiodothyronine (T3) may also be correlated with malignancy as well. Cho et al has shown that free T4 (fT4) is elevated in patients with thyroid cancer (odd's ratio (OR) of 1.73) [19]. However, the inverse has been shown to be true for total T3 (TT3) by Jonklaas *et al*[20]. Rinaldi *et al* [21] who non-significantly found high fT3 to be indicative of cancer risk. Given that this study has not found any evidence in support of either group, it is possible that it was not sufficiently powered to find such a correlation, or that no relationship exists between the two.

Conclusions

There was a highly significant relation procalcitonin and thyroid cancer specially medullary type.

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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Detection of Klebicin Gene Cluster from *Klebsiella Pneumoniae* Isolated from Sputumat Baghdad City

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Abstract

Klebicins (bacteriocins) are natural antimicrobial peptides that produced by ribosomes of *Klebsiella* species. The fatal action of klebicin is directed to closely related bacteria to the producing strains. The present study aims todetect theklebicin genecluster in *Klebsiella pneumoniae isolates*.Thirty one *K. pneumonia*isolates were specified by biochemical and microbiological methods, eventually confirmed by API 20E system and VITEK-2 system.Antibiotic susceptibility test was done five antibiotics (Amikacin, Ampicillin, Augmentin, Azetreonam and Tetracycline). DNA extraction was carried out for 31 *K. pneumonia* isolates andklebicingene cluster was amplified from extracted DNA by PCR technique. Results of current study were shown that the resistance rate of studied antibiotics was registered as Amikacin 32.26%, Ampicillin 64.52%, Augmentin 51.61%, Azetreonam48.39% and Tetracycline 41.94%.Amplification results revealed that Klebicin gene cluster was isolated from15(48.39%) of *K. pneumoniae* isolates.

Keywords: *Bacteriocin,Klebicin, Antibacterial agents,Klebsiellapneumoniae.*

Introduction

K. pneumoniae is Gram-negative, non-motile encapsulated rod-shaped bacterium, facultative anaerobic andlactose-fermenting[(1)].*K. pneumonia*is a dangerousopportunistic pathogen due to their role in causing both nosocomial andcommunity acquired infections[(2)].In healthy human, it can be situatedasymptomatically in the intestine, skin, nose, and throat. On the other side, it can cause a large spectrumof infections including mostly pneumonia, wound, soft tissue, or urinary tract infections[(3)].Becausethey survive in the environment and require their competition with other microorganisms, whole bacteria yield antimicrobial peptides act as inhibitor or killer for other competing bacteria. These peptides are included two groups, the bacteriocins and the antibiotic peptides[(4)]. Since 1925, the first bacteriocin in *Escherichia coli* was discovered by Andre Gratia. In 1963, Hamon and Peron were first described bacteriocins of *Klebsiella*[(5)].

Klebicins or klebocins (Bacteriocins)are ribosomally synthesized natural antimicrobialpeptides. These peptides are excreted by most bacteria for killing other bacteria[(6)].They differ from classical antibiotics in having fatal action against only bacteria which are closely linked to the producing strains[(5)]. The action of Klebicin includes several steps for killing the sensitive bacteria. These steps starting with binding of klebicinto specific receptors, which are outer membrane proteins utilized for the entry of particular nutrients then transport through the outer membrane and pass across the periplasm by either the Tol or TonB systems[(7)]. Klebicins would reach their target and their action would do either by forming a voltage-dependent channel into the inner membrane or by utilizing their endonuclease activity on DNA, rRNA or tRNA[(8)].Klebicins were proteins encoded chromosomallyand byKlebocinogenic plasmid[(9)].

The present study aimed to detect klebicin (bacteriocin) genecluster among *K. pneumonia*isolated from sputum in Baghdad city.

Materials and Method

Samples Collection: Thirty one isolates of *K. pneumoniae* were isolated from sputum samples collected from patients suffered from pneumonia admitted at Baghdad Medical City (Baghdad Teaching Hospitaland Gazi Al-Hariri Hospital) during the period

from January/ 2015 to May/ 2015.

Identification of Isolates: The identification of all *K. pneumonia*isolates were carried out by inoculation on blood agar andMacConkey agar for primary identification. They were incubated aerobically at 37˚C for overnight. All *K. pneumonia*isolates were specified to genus and species level based on the standard biochemical and microbiological methods[10]. API-20 E system wasutilized to affirmthe identification[11]. The confirmative testshave been crowned byVITEK-2 System.

Antimicrobial Susceptibility Test: Antimicrobial susceptibility test was achieved against five antibiotics (Amikacin, Ampicillin,Augmentin, Azetreonamand Tetracycline) by using the disk diffusion methodand the outcomes were interpreted in based on the CLSI, 2014[12].

Amplification of klebicingene cluster: DNA extraction was carried out for 31*K. pneumonia*isolates by using ZR Fungal/Bacterial DNA MiniPrep™ kit.The klebicingene cluster was amplified from extracted DNA. A 2300bpklebicin gene cluster was amplified using the primer forward (5'-GCTCTGTAACCTTCAAGTTCTC-3') and reverse (5'-CAAGCAAGATTACGGTCTACTC-3')[8]. The PCR reaction mixture was composed of 12.5µl of green master mix, 3µl of template DNA, 2µl of each forward and reverse primer (10µM), then the volume completed to 25µl by free nuclease water.The PCR program was applied as the following: 10min at 94˚C (initial denaturation temperature);32 cycles including 1min at 94˚C (denaturation temperature), 40S at 54˚C (annealing temperature) and 2min at 72˚C (extension temperature);and 5min at 72˚C for finalextension. Amplified DNA productswere resolved by electrophoresis on 1% agarose gels containingRedSafe™ Nucleic acid staining.

Findings and Dscussion

K. pneumoniae is classified as most important "Gram negative bacteria"[13], because their pathogenic role in hospital acquired infections[14]. In the past decades, thepersistentrising in the incidence of bacterial infections has led to continuous and unlimited utilization of antimicrobial drugs as therapythroughout of the world. Subsequently, this cause increasing the emergence of "multidrug resistance" among *K. pneumoniae* and other strains of bacteria[15].

Antimicrobial susceptibility testing has givenvariouslevel of resistance against antibiotics as appeared in Table1.There is an evidence for increasing the antibiotic resistance.The higher percentage of resistance was recorded against ampicillin 64.52%,followed by augmentincame in second-step with 51.61%. On the other hand, the percentages of resistance against azetreonam and tetracyclinewere convergent (48.39%and 41.94% respectively), while the percentage was 32.26% for amikacin resistance. *K. pneumoniae* resistance to various antibiotics as discussed, this is thought to be in relation with Kaye *et al*[16].

Table 1: Antibiotic Resistanceamong*K. pneumoniae* isolates (n=31).

Antibiotics	**Resistant *K. pneumonia***	
	No.	**%**
Amikacin	**10**	**32.26%**
Ampicillin	20	64.52%
Augmentin	16	51.61%
Azetreonam	15	48.39%
Tetracycline	13	41.94%

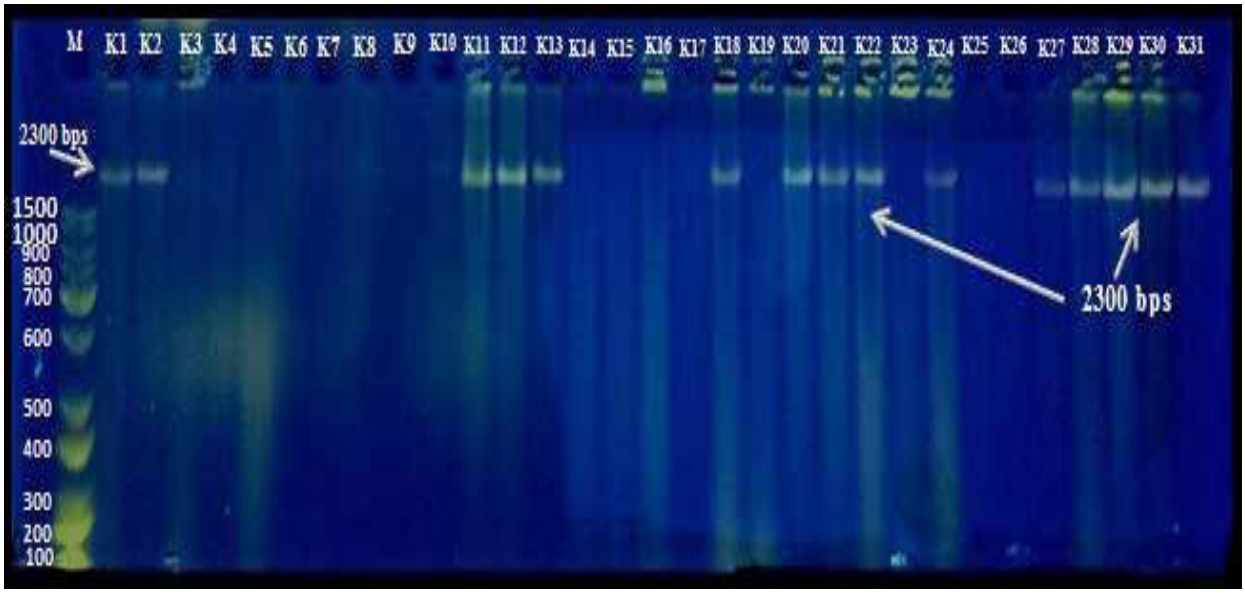

Figure (1): Analysis of *Klebicin gene cluster* of *Klebsiellapneomoniae* isolates. Lane(M): 100bp DNA Marker. Lane(K1,K2,K11-K13,K18, K20-K22, K24,K27-K31):*Klebsiellapneomoniae* isolatespositive for*Klebicin*gene cluster. All positive isolates for *Klebicin*gene clusterare generated (2300bp) PCR product.

Amikacinusually act bybinding to 30s ribosome leading to misreading of mRNA, eventually inhibiting of bacterial protein synthesis [17]. The study findings showed that ten *K. pneumoniae* isolates were resistant to amikacin with percentage (32.26%) which is close to the result (28.12%) that reported by Aljanaby and Alhasani, (2016) in Kufa city [18]. This result is lower than other studies documented by Bratu*et al*, 2005 (45%) and Castanheira*et al*, 2008 (53.3%) [19,20].Ampicillin belong to one group of "beta-lactam antibiotics" and consider as a member of the amino-penicillin family.The action of ampicillin occursby inhibition of the transpeptidase enzyme, therefore it categorized as cell wall inhibitor for bacteria [21].In present study, most isolates showed high degree of resistance to ampicillin (64.52%). These findings were in agreement with those reported by Alain *et al*[22], and Kevin *et al*[23].The study showed that 16(51.61%) of the isolated*K. pneumoniae*recorded resistance againstAmoxicillin/Clavulanic acid. Another result was documented in a study carried out byAljanaby and Alhasani (2016) in Iraq, on the *K. pneumoniae* isolates showed resistance (93.75%) to this antibiotic [18].*K. pneumoniae* isolates were showed high resistance to azetreonam. Fifteen*K. pneumonia*isolates (48.39%) were resistant to azetreonam. This prevalence rate ofazetreonam resistance was higher than these reported from 1998 to 2010 by Sanchez *et al*[24].

Tetracycline are a broad group of antibiotics that was isolated from different species of the genus Streptomyces. Tetracycline was discovered in 1945 by Benjamin Duggar[25]. Tetracycline act as inhibitor for protein synthesis by binding to the rRNA components of 30S ribosomal subunit and leading to inhibit the addition of aminoacyl-tRNAs to the ribosome [26].The study recorded that the tetracycline resistance was 41.94% which was higher than result reported by Aljanaby and Alhasani (2016), who found that the tetracycline resistant *K. pneumonia*was 34.37% [18].

The klebicins actionis homologous activity due to lethal action on limited spectrum of bacteria which are closely related to the producing isolates [27], and they have a broad antimicrobial spectrum that not limited by the genus and family [28]. Klebicins are divided into two types, A and B, according to cross resistance [29], their action on the goal cell, causing pores in the outer membrane of the target cell making ionic channels in it. The other endonucleases klebicins hydrolyze the nucleic acid of the target cell in the cytoplasm.Just two bacteriocins [klebicins B [30] and CCL (NCBI accession AF190857)] from *Klebsiella*have been sequenced[30]. Klebicin gene cluster among 31 of *K. pneumoniae*isolates were conducted using specific primers.Figure (1) shows the result of PCR amplification. PCR products corresponding to Klebicin gene cluster (2300bps) were appeared in 15 isolates (48.39%).

Ethical Clearance: Non

Source of Funding : Self

Conflict of Interest : None

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The impact of ZnO Fillers on the Tensile Strength of Self-Polymerizing Acrylic Resins Specific for Orthodontic Appliance

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Abstract

Background : self-polymerizing acrylic resins are the common materials used for the construction of orthodontic appliances. These materials have to be deficient some mechanical properties. The aim of the present research was to assess the impact of adding zinc oxide fillerstoself-polymerizing acrylic resins on their tensile strength.**Materials and method**: thirty samples were fabricated in total. They were made from self-polymerizing acrylic resin. 20 specimens were strengthened with zinc oxide powder at 1% and 2% by weight respectively, and the other ten specimens were considered as control. The tensile test was conducted for each specimen. The data were analyzed using SPSS v 20, and comparison between main groups was obtained by ANOVA and Duncan's test. **Results**: The results showed that the ZnO enhanced significantly the tensile strength of self-polymerizing acrylic resin compared to the control group. The ZnO 1 experimental group has the highest mean of tensile strength (6.97 N/mm2) followed by ZnO 2(5.91 N/mm2).**Conclusion**: the incorporating of ZnO powder to self-polymerizing acrylic resins affects significantly the tensile strength. It is suggested to add ZnO fillers with different concentrations to see whether there is any improvement in the mechanical properties of self-polymerizing acrylic resins or not.

Keywords*: self-polymerizing, Zinc oxide, tensile strength.*

Introduction

In dentistry, self-polymerizing acrylic resins are the most common restorative substances used for fabrication of orthodontic appliances [1, 2]. The advantage of such materials is that usually cheap and easy to be manipulated, and being chemically activated [3, 4]. However, the degree of polymerization of these materials is not perfect as heat polymerizing acrylic resins, results in a high unreacted residual monomer leading to lowering flexural strength of acrylic resin [5]. To overcome such problems, researches have been carried out by adding several reinforcements (i.e. titanium dioxide) to enhance the mechanical properties of acrylic resins. It was found in the literature that the addition of fillers enhanced the mechanical properties of acrylic resins [6]. However, others found that the addition of fillers has a negative impact on some mechanical properties of acrylic resins[7]. Zinc oxide powder is found as the mineral zincite and can be produced synthetically. It is white in colour with a formula of ZnO, which can insoluble in water [9]. The zinc is found in pure form as brittle metal and it is a white-blue coloured metal. The zinc, when heated, oxidizes results in a low-density metal oxide [10-11]. In dentistry, Zinc oxide has utilized in wide applications (i.e. root canal sealer) [12-13]. The present research was to assess the influence of incorporating ZnO fillers to self-polymerizing acrylic resins on their tensile strength

Materials and Method

Materials and samples groups:

In the present study, 30 Samples were prepared in total. There are 3 main groups and each group had ten samples. The experimental groups involved the adding the zinc oxide powder (Golchadent, Iran) with a concentration of 1% and 2% respectively. The control group involved fabrication of self-polymerizing acrylic samples (Spofadental, Czech Republic) without the addition of zinc oxide filler. The dental stone (Zhermack,

Italy) was utilized to fabricate the mould for acrylic samples.

Fabrication of self-polymerizing acrylic samples

A metal pattern with dimension of 65mm length, 12.5mm width and 2.5mm thickness was utilized to construct the acrylic samples for tensile strength test [14]. The specimen's preparation and procedure were done depending on ADA specification number 12 for denture base resin (1999) [15]. The process of fabrication of stone mould began by lubricating the lower part of the metal flask with a Vaseline to allow removal of stone mould following deflasking. The lower part was then filled with dental stone, and the metal pattern was placed gently into mid of stone surface with taking into consideration half of its thickness was exposed (figure 1).

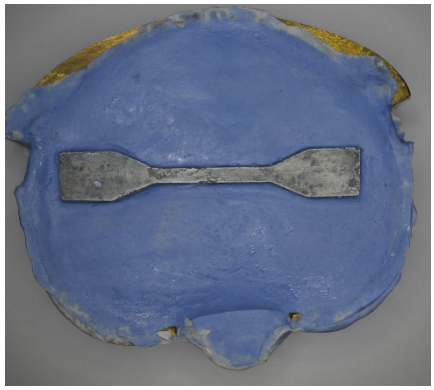

Fig1.Metal pattern located onto stone mould

The stone surface was left to set for one hour. It was then coated with a separating medium (ISODENT, Spofadental, Czech Republic). The upper half was separated with a Vaseline and filled with dental stone completely; the lower half was inverted over upper half gently and left to set for one hour. Then, the flask was carefully opened; and the pattern was taken away in order to be prepared for packing the acrylic resins.

Mixing and proportioning of acrylic resins:

In the present research, an electronic balance with an accuracy of (0.0001g) was used (fig 2). The amounts of acrylic polymer, monomer and ZnO nano-fillers are shown in table 1.

Table1. Proportioning and Mixing of the Acrylic resin

ZnO Percentage	Amount of ZnO.	Amount of PMMA.	Amount of monomer.
1%	0 .22 g	21.78 g	10 ml
2%	0.44 g	21.56 g	10 ml

Fig 2.An electronic balance used in the current study

According to manufacturers' instructions, the materials were hand -mixed in a clean and dry ceramic jar for 30 seconds. The mix was then left at the room temperature for 6 min. When it reached a dough stage, the acrylic dough was rolled and positioned in the mould. Then,the two halves of the flask were put in contact and positioned under press (up to 20 bar). Then the two halves of flask wereseparated and the excess materials were removed with wax knife. The two halves of the flask were put under pressure till metal to metal contact and left-handed under press for five minutes. The stone bursand sandpapers (120 grain size) were utilized to get a smooth surface. A bristle brush and pumice were used for polishing the acrylic samples. A wool brush and polishing soap were utilized to get a glossy surface. The specimens were conditioned for two weeksat (37°C)in distilled water [15].

Testing procedure: -

The Instron machine (Instron/ Germany) was used to test Acrylic samples (figure 3).

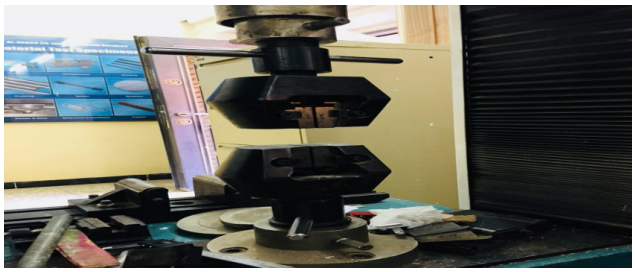

Fig3. Specimen under tensile strength test.

All acrylic samples were subjected to the force with a crosshead speed of 0.5 mm/min until fracture occurred and the values were recorded in kilograms (Kg) and then converted into Newton (N)[17]. Tensile strength values were then calculated by the formula below [18]:-Tensile strength(T.S) (N/mm2)= force at failure(F) / the area of cross-section at failure (A)

Results

The values of Mean and Standard deviation (SD) are listed in the table (2). The results indicated that the ZnO enhanced significantly the tensile strength of self-polymerizing acrylic resin compared to the control group. The ZnO 1 experimental group has the highest mean of tensile strength (6.97 N/mm2) followed by ZnO 2(5.91 N/mm2).

Table 2. Mean, standard deviation of groups for tensile strength.

Group	Number	Subset for alpha = 0.05		
		1	2	3
Control	10	5.18		
ZnO 1	10		5.91	
ZnO 2	10			6.97
Sig.		1.000	1.000	1.000

Furthermore, there is significant difference amongall group (P<0.001) as shown in Duncan multiple comparison test (table 3).

Table 3. comparison between the groups

group	mean	Standard deviation
control	5.18	.51
Experimental group ZnO 1	5.91	.76
Experimental group ZnO 2)	6.97	.12

In addition, there were significant differences among all samples groups(P<0.001)as illustrated in table 4(ANOVA test).

Table 4.ANOVA test.

ANOVA					
tensile					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	16.183	2	8.092	28.512	.000
Within Groups	7.662	27	.284		
Total	23.845	29			

Discussion

The present study has been conducted to evaluatethe influence of adding ZnO fillers to self-polymerizing acrylic resins ontheir tensile strength. In the literature, there were no published papers about the effect of ZnO fillers on mechanical properties of the self-polymerizing acrylic resins. The present study indicated that the addition of ZnO micro filler enhanced significantly the tensile strength shown in the table (3). The rise in tensile strength values following incorporating 1% and 2% of ZnO powder is because of good distribution of particles enable them to inter between liner macromolecules chains. The segmental motion of the macromolecular chains was restricted and lead to increase strength and rigidity of the resin. Consequently, this enhanced the

fractural resistance and lead to improve tensile strength [19,20].

Conclusion

From the present study, it is concluded that the incorporation of zinc oxide improves slightly the tensile strength of self- polymerizing acrylic resins. It is suggested to do more research using the ZnO fillers with different concentrations to see if there is any improvement in the mechanical properties of self-polymerizing acrylic resins.

Conflict of Interest: No

Source of Funding : Self

Ethical Clearance : Non

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Hormonal Changes in Sodium Fluoride Exposed Female Rats and its Amelioration by *Urticadioca* Leaf Extract

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Abstract

The present study was carried out to investigate the protective role of *Urticadioica*leaf extract on the hormonal profile in sodium fluoride exposed female rats. Thirty two mature female rats were randomly divided to four equal group and treated for 4th weeks as follows: control group administered tap water; group T1 administered tap water containing 100 ppm NaF; group T2 administered tap water containing 100 ppm NaF plus 300 mg/ kg *Urticadioica*leaf extract; group T3 administered 300 mg/ kg *Urticadioica*leaf extract only. Blood sample were obtained at zero and 28 days of the experiments for estimation of serum progesterone, estrogen, FSH,LHconcentration. The result revealed significant decrease in serum progesterone, estrogen, FSH, LH concentration in group T1 (NaF treated group). On the other hand, the result showed the beneficial effects of *Urticadioica* leaf extract to improvement the previous parameters against NaF(T2), through a significantincrease in hormonal profile concentration. Therefore, it could be conclude that *Urticadioica*extract has a potent ameliorative effects on hormonal profile in NaF stressed rats .

Keywords: *Hormonal profile, sodium fluoride, urticaleaf, rats*

Introduction

Fluoride surely exists in water, soil, and nutrition. Ingesting water that have high concentricity of fluoride, mainly from natural origin, is the major source of human environmental exposure throughout the world[1]. Fluoridation is a safe and efficient method for the preventing of dental caries[2,3]. In several countries, water fluoridation is used for this reasons. However, the advantageous will be narrow, and healthiness may be affected adversely if excessive fluoride be absorbed. Fluoride accumulation in soft tissues causes oxidative stress through inhibition of different enzymatic systems and increased generation of reactive oxygen species (ROS)[4]. As the case of the many chronic degenerative disease, increase of ROS and lipid peroxidation (LPO) has been considered to play an important role in the pathogenesis of fluoride toxicity[5]. NaF may have toxic effects on the brains of suckling mice[6], may impair learning and memory in rats[7,8],and may impairment of liver function[9].

Excessive exposure to environmental pollutants and chemicals is a main principle to reproductive health problems[10,11]. Fluoride is a common natural pollutant with certain toxic effects, and the association between long term fluoride exposure and fertility impairment has be attracted to concern[12,13].

It is well known that folk medicine was assistance in disease prevention and control have been attributed to antioxidant properties of their constituents, broadly termed as polyphenolic compounds. In addition to their role as antioxidants, these compounds have broad spectrum of medical property. It has been known that antioxidants protects the cell directly or indirectly from the damage caused by toxic radicals reactions through different mechanisms[14].*Urticadioica*belongs to the Urticaceaeitsthe chemical compounds are Lignans, sterols,flavonoids, polysaccharides, lectins and fatty acids also rich in vitamins A,C and D and such minerals as Magnesium and Calcium.*Urticadioica*has an antioxidants, anti inflammatory and anticancer properties, as well as, blood glucose and lipid lower effects[15]. Different effects of it plant have been notified in various worldwide districts. Therefore, the objective of this study was to explore the protective effect of *urticadioica*leaf extract on hormonal profile against sodium fluoride in female rats.

Materials and Method

The leaf 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[16,17], the extract have been filtered 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.32adult female rats were randomly divided to 4 equal group and handles as follows for 4th weeks: control group were administered tap water; group T1rats of this group were allowed to *ad libitum* supply of drinking water containing 100 ppm of sodium fluoride**;**group T2rats of this group were allowed to *ad libitum* supply of drinking water containing 100 ppm of sodium fluorideplus ethanolic extract of*Urticadioica* (300 mg/kg)**,** groupT3rats of this group were receivedEthanolic extract of*Urticadioica*at dose of 300 mg/kgonly**;**Collected of blood sample were obtained via cardiac puncture from each anesthetized animal at zero and28 days of the experiments then centrifuged at 3000 rpm for 15 minutes, and sera was isolated and frozen at -18C° till analysis of serum estrogen,progesterone,FSH, LH concentrations by using immunoenzymometric assay kits (monobindInc, USA). Statistical analysis of data was performed on the basis of two way analysis of variance (ANOVA)using a significant level at($P<0.05$) and using Least Significant Differences (LSD)test for specific group differences[18]**.**

Finding and Discussion

Data pertaining to serum progesterone hormone concentration (table 1) showed a significant($P<0.05$) decline in NaF treated group after 4thweeks of experiments comparing to other treated groups.The results also clarified a significant($p<0.05$) increment in T2 as compared to T1 treated group. It was also noted highly significant ($p<0.05$) increase in this parameter in T3 group comparing to other treated groups.

In comparison within time for T2&T3 groups, recorded significant($p<0.05$) increment, whereas T1 group registered significant ($p<0.05$) decrement as compared to pretreated period.

Table 1:effects of alcoholic extract of *Urticadioica*on serum progesterone hormone concentration (ng/ml) in adult female rats exposed to sodium fluoride

Time Group	C	T1	T2	T3
Zero day	2.50±0.005 A a	**2.50±0.00** A a	2.48±0.01 A b	**2.49±0.01** **A b**
4th week	2.49±0.01 C a	1.17±0.00 D b	3.19±0.00 B a	3.29±0.01 A a

LSD 0.02 Value are expressed as mean ±SE, n=8 each ;C:control ;T1: administration 100 ppm of NaF;T2: administration 100 ppm of NaF plus300 mg/ kg*urticadioica* extract;T3: administration 300 mg/ kg*urticadioica* extract treated group; capital letters denotes differences between groups,$p<0.05$ and small letters denotes significant differences within group $p<0.05$

After 4th weeks of experiments, group T1 showed highly a significant ($p<0.05$) decrease in estrogen hormone concentration compared to other treated groups (table 2). Whereas, the result registered that oral intubation of extract concurrently with NaF normalized estrogen hormone near to control group after 4th weeks of treatment. On the other hand,estrogen hormone concentration in T3 group recorded significantly ($p<0.05$) elevated after 4th weeksof experimental periods comparing to other treated groups. Time dependent, a significant($p<0.05$) increment was observed after 4th weeks of treatment in T3 group, also a significant($p<0.05$) decrement in C,T1,T2 treated groups when compared with zero time.

Table 2:effects of alcoholic extract of *Urticadioica*on serum estrogen hormone concentration (ng/ml) in adult female rats exposed to sodium fluoride

Time Group	Control	T1	T2	T3
Zero day	**27.17±0.33** **A a**	**27.06±0.21** A a	27.40±0.25 A a	**27.14±0.08** **A b**
4th week	25.80±0.68 B b	21.52±0.45 C b	25.48 ±0.45 B b	32.66±0.93 A a

LSD 1.1

Note for details show table 1

Table(3)showed a significant($p<0.05$) decrement in FSH concentration NaFtreated group comparing to controlgroup.Whereas, highly significant increment in this parameter was observed in T3 group as comparing to other treated groups. Besides, there were a significant($p<0.05$) increase in FSH concentration in C,T2,T3 groups, while results showed a significant($p<0.05$)decrease in T1 treated group at end of experimentscomparing to zero time.

Table 3: effects of alcoholic extract of *Urticadioica*on serum follicular stimulating hormone concentration (FSH) (ng/ml) in adult female rats exposed to sodium fluoride

Time Group	Control	T1	T2	T3
Zero day	12.96±0.24 A b	13.12±0.19 A a	13.07±0.19 A b	12.94±0.23 A b
4th week	13.46±0.38 B a	10.41±0.19 C b	13.73±0.23 B a	16.95±0.19 A a

LSD 0.4

Note for details show table 1

Statistical analysis of LH concentration recorded a significant ($p<0.05$) decrease in groupT1comparing with other treated groups table 4.On the other hand, significant ($p<0.05$) elevation in same parameter in T3 treated group when comparing with other groups. Furthermore,there was no significant ($p>0.05$) differences in LH concentration in control &T2 compared with eachothers.Moreover,with exception of T3(which showed a significant ($p<0.05$)increase after 4th weeks), the results recorded a significant ($p<0.05$) decrease in C,T1 and T2 along the experiment period comparing to pretreated period.

Table 4: effects of alcoholic extract of *Urticadioica*on serum luteinizing hormone concentration (LH) (ng/ml) in adult female rats exposed to sodium fluoride

Time Group	Control	T1	T2	T3
Zero day	4.27±0.03 A a	4.32±0.02 A a	4.36±0.02 A a	4.34±0.02 A b
4th week	3.73±0.23 B b	2.81±0.23 C b	3.86±0.27 B b	6.15±0.31 A a

LSD 0.4

Note for details show table 1

The ovary it is an prominent target organ of many reproductive toxicants. Organ coefficients can better express organ toxicity follow exposure at toxic chemicals. Exposure to NaF is associated with deterioration of reproductive system organs functions specifically the Gonads (ovaries)[19]. The results have exhibit that NaF causes a significant ($P<0.05$) drop off in estrogen & progesterone concentration (T1) compared with control group, this reduction may produce direct effect of NaF on ovarian tissues specially the granulosa cells that have specialized receptors for FSH hormone, in order that these cells does not response to FSH hormone and the release of estrogen will be decrease[20]. To maintenance of fertility investigations have confirmed that steroid hormones, such as estrogen &progesterone, role in the growth and differentiation of reproductive tissues[21]. Estrogen can increase the sensibility of granulosa cells to FSH and LH, through rising the biosynthesis of progesterone by granulosa cells[22], E2 modulates steroidogenesis, promotes granulosa cell proliferation,and maintains follicular development[23]. Beside, NaF that caused significant($P<0.05$) decrease in cholesterol which considered as precursor of steroid hormone may be the second cause of this reduction in estrogen and progesterone concentration[24].

Its well-known that the secretion of reproductive hormones from the ovary is regulated by the release of LH and FSH from the anterior pituitary gland. Based on the reproductive the results obtained in this study, showed that FSH and LH secretion was significantly inhibited in the sodium fluoride-exposed groups[25]. The main function of FSH is to stimulate ovarian growth and promote follicular development. LH participates in ovarian regulation, plays a critical role in follicular maturation, ovulation, and corpus leutum development, and intervenes in the synthesis of steroid hormones[26]. As well, the present study the prevalent mechanism of action of the harmful effects of NaF occurs at the level of the hypothalamus–pituitary axis may be indicates that NaF inhibits the secretion of FSH and LH from the pituitary gland, thus weakening the promotional activity of FSH and LH on ovarian granular cells.

On the other hand, the result exhibited that the elevation of steroid hormones progesterone and close control group of estrogen hormone after treatment with NaF and *Urticadioica*(T2) may be due to role of plant extract to reduce the negative effects of toxicity of NaF[27]. Lignans, sterols and beta sitosterol are identified phytoestrogens in *Urticadioica*[28,29]. They suggested that nettle can be used in diet as an appropriate pharmaceutical alternative for synthetic prescription of estrogen hormone in patients with osteoporosis[29], besides, nettle extract improves levels of such minerals as calcium and magnesium that were decreased in patients with osteoporosis.Calcium plays a major role in reproduction. Deficiency in this macro mineral can affect fertility[30]. Calcium-dependent reproductive mechanisms include steroid biosynthesis in adrenal glands and ovaries. Calcium may also affect delivery of cholesterol or using cholesterol in mitochondria or stimulating conversion of pregnenolone to progesterone in steroidogenesis[31,32].

From results obtained it can be explained that *Urticadioica* have an important role in elevation of LH and FSH level. This elevation may be due to the active constituents of *Urticadioica* in activation of synthesis mechanisms of these hormones by stimulation of hypothalamus or pituitary glands to release and secretion

gonadotropin hormone[27].On the other hand, the increase of LH and FSH hormones may be due to the high constituents of *Urticadioica*from essential nutrients such as flavonoids, sterols, polysaccharides,fattyacid, also rich in vitamins and minerals,theseantioxidant nutrients may stimulate hypothalamus and pituitary gland to increase synthesis and release of these hormones[34].The increase of FSH and LH hormones in(T2) group compared with control group may be increase the follicular growth so that there were increase in the number of Graffian follicles which increase the synthesis and release of estrogen hormone [34]. Also the increase of LH hormone in (T2) group compared with control may be increase the level of estrogen because the high level of LH hormone leads to increase of its binding with the receptors on theca cells[35].

The increase of LH hormone is considered as a principle factor to stimulate the theca interna to elevate production of pregnenolone compound which converted by granulose cells to progesterone[35]**.** Also the increase of progesterone may be due to the role of LH hormone to stimulate the corpus leutum to production of progesterone[34]. In conclusion *Urticadioica*extract has a potent ameliorative effects on hormonal profile in NaF stressed rats.

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Funding –Self

Ethical Clearance- With agreement

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Application of Silver Nanoparticles (Silver NPs) in Assisted Reproductive Techniques (ART) as a Carrier Vehicles to Improve the Therapeutic Influence of Doum Extract for Sperm of Asthenozoospermic Patients

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Abstract

Assisted reproductive techniques are a tool practiced worldwide to help couples achieve pregnancy and preserve fertility. However ART reveals several limitations which needs for new alternatives to overcome these limitations. Over recent years, nanotechnology has been applied as a tool to improve strategies to enhance the outcome of ART. This study has investigated for the first time the impact effect of implication of silver NPs to boost therapeutic effect of Doum palm (*HyphaeneThebaica*) extract on enhancing sperm quality of asthenozoospermic patients. The findings of this study has showed the implication of silver NPs has significantly enhanced theSuperoxide dismutase activity, and reduced Malondialdehyde expression and sperm DND fragmentation percentage. These encouraging results suggest that nanotechnologies could be easily included in the routine procedures of ART to improve sperm quality and thus increase the rate of successful pregnancy and preserve fertility.

Keywords: *Asthenozoospermia, Silver nanoparticles, Doum palm (HyphaeneThebaica), Extract.*

Introduction

Asthenozoospermia (or asthenospermia) refers to decreases of sperm motility, which is one of the major causes of infertility or reduced fertility in men.Oxidative stress plays important role in causingdecreases of male fertility and has a significant roleon semen quality assessment. Oxidative stress is mainly caused as a result of accumulation of excessively reactive oxygen species (ROS) or reactive nitrogen species (RNS). ROS/RNS results in severe damage of sperm DNA, reduced sperm motility, reduce sperm fertilising ability, and decrease sperm membrane integrity via lipid peroxidation; all of which are underlying mechanisms lead to sperm dysfunction [1].

In reproductive medicine, the application of medicinal herbs has extensively investigatedfor their pharmacological therapeutic activities on improving sperm quality. Many medicinal plants have been reported to have beneficial effects in combination with assisted reproductive technology (ART) to increase the chances of pregnancy rate and reduce the cost of treatment. *Hyphaenethebaica* which, belongs to the family Palmae and this plant is commonly known as a Doum palm, has reported to has many medicinal therapeutic activities[2, 3]. In these studies, fruits of Doum showed antimicrobial, anti- oxidant, hypolipidemic, antidiabetic, and antihypertensive activities, suggesting that the active biological effectsof Doum fruits may ascribed to its active components [3, 4].

In fact, ART has been highly applied and practiced worldwide as a set of methodologies to cases related to infertility. However, ART still has some challenges, which still needparticular focus[5].Therefore, it is highly important for identifying new alternatives to overcome these challenges.Recent advances in nanotechnology have encouraged the application of nanoparticles (NPs), which has already been developed for non-human animals, in assisted reproduction in humans[5-7]. Nanoparticles characterised by various physical and chemical properties that make them more stable, soluble and more biologically effective to employed in the field of drug delivery. In fact, silver NPs are type of metallic NPs and have been frequently used in many

biomedical application due to their unique physical, chemical, and biological properties[8-10]. In fact, there is a little information is available about their activity in the reproductive system. Therefore, this study has adapted innovative different strategy to improve male fertility diagnosed with asthenozoospermia, particularly by enhancing sperm cell quality using silverNPs as a carrier vehicles for Doum extract components.

Materials and Method

Preparation dried extract of Doum palm (HyphaeneThebaica)

The Doumpalm fruit was dried and grinded, once it has been obtained from the local market. A 20gm of Doumpalm fruit powder was mixed with 100ml of distilled water and refluxed for 3hours. As 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 73ºC [11].

Preparation of aqueous Doum palm (HyphaeneThebaica) extract for in vitro sperm activation

Following complete drying of extract of Doum palm, the aqueous working solution of Doum extract was prepared by dissolving 0.5mg of dried Doum extract with 10ml of phosphate buffer solution (PBS) (Sigma-Aldrich, USA) in plastic test tube (Falcon, USA) contained broad spectrum antibiotic. TheDoum aqueous working solution has prepared at 20% concentration by adding 2ml of the aqueous working solution of Doum extract to 8ml of PBS,to be used for the purpose of in vitro sperm activation experiment. The solution was filtered using Millipore (Millipore, USA) 0.45µM. Media was adjusted at pH 7.5-7.8 and stored at 25ºC.

Mixture preparation of silver nitrate nanoparticles with aqueous Doum extract for in vitro sperm activation

The aqueous Doum extract was mixed with silver nitrate nanoparticles by adding 0.58mg of silver nitrate to 50ml distal water. Then 10ml of aqueous Doum extract was added to the 50ml of silver nitrate solution. The final mixture of aqueous Doum extract and silver nitrate nanoparticles kept on magnetic stirrer until the brown colour of mixture changed to the brown red colour.

Experimental design

Semen samples were collected from ten patients diagnosed with asthenozoospermia, following the ethical approval and informed consent from all donors. Each semen sample was divided into three groups. The sperms in first group (i.e. control group) were treated with PBS, sperms in second group were treated Doum extract only (Extr. alone group) and sperms in third group were treated with Doum extract with silver NPs (i.e. Extr. +NPs group). Samples were incubated for 1-2hours at 37ºC in an incubator for further experiments.

Superoxide dismutase (DOS) activity assay

Using the commercially available colorimetric assays (Cohesion Biosciences. LTD. China) and following the methodology provided by the manufacturer DOS was detected in all sample groups. Briefly, sperm samples at 5x 10^6 were centrifuged. Following centrifugation, the supernatant was discarded and 1 ml of assaybuffer was added. Then, samples were sonicated (with power 20%, sonication 3s and intervention 10s, repeated 30 times) and centrifuged at 8000g 4°C for 10 minutes. Following centrifugation the supernatant was moved into a new centrifuge tube and kept on ice for further processing. 19µl of samples, stander and distilled water as (blank) were added into 96 well plate. Then, 30µl of reaction buffer, 100µlsubstrate, 1µl of enzyme and 50µldye reagent were added to samples, stander and blank. Finally, the plate was incubated at room temperature for 30 minutes and absorbance measured at 560nm.

Malondialdehyde (MDA) measurement assay

Using the commercially available colorimetric assays (Cohesion Biosciences. LTD. China) and following the methodology provided by the manufacturer MDA was detected in all sample groups. Briefly, sperm samples at 5x 10^6 were centrifuged. Following centrifugation, the supernatant was discarded and 1 ml of assay buffer was added. Samples were sonicated (with power 20%, sonication 3s and intervention 10s, repeated 30 times) and centrifuged at 8000g 4°C for 10 minutes.Following centrifugation the supernatant was placed into a new centrifuge tube and kept on ice for further processing. 100µl of samples were added into new micro centrifuge tubes with 200µl dye reagent and placed into oven with 90ºC for 30 minutes. Then, micro centrifuge tubes were placed on ice for few seconds and centrifuged at 10000g, 25°C for 10 minutes. 200µl of samples supernatant were

added to 96-well microplate absorbance measured at 532nm or 600nm.

DNA fragmentation assay

The assessment of sperm DNA normality was performed usingthe microscopicacridine orange (AO) test following the protocol described previously in 1984 by Tejada et al. [12]. Briefly, Smears of10 semen samples were fixed in Carnoy's solution (3:1,methanol:glacial acetic acid) at 4°C and left overnight , washed and air-dried. The slides were then immersed in AO working solution (0.19 mg/ml in 0.1 M citric acid and 0.3 Na2HPO4 7H2O, pH 4) for 10 minutes. Smears were assessed on the same day using fluorescent microscope (Zeiss Co., Germany) with a 460nm. Normal DNA (double stranded)emitted green fluorescent when stained with AO,whereas the stain emitted red fluorescent when it bind to fragmented DNA (single stranded).

Statistical analysis

GraphPad Prism 8.0.2 software was used for data analysis. Data of N=10 was tested for normal distribution using three different tests D'Agostino& Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test.

Finding

According to The World Health Organization (WHO), couples may classify as infertile couples if they fail to achieve a clinical pregnancy after, at least, 1 year of regular unprotected sexual intercourse[13]. The application of ART methodologies to cases related to infertility is common and practiced worldwide. However, there are some challenges, which still require special attention[5].

Over the recent years, nanotechnology has been considered to help overcome some of challenges in reproductive medicine. NPs are owning unique physical and chemical propertiesmaking them a valuable and useful materials for various applications[5, 14]. The recent advancing in nanotechnologies have increased their possible applications in ART methodologies.

This study has investigated for the first time the influence of implication of silver NPs on improving the effect of Doum extract for enhancing sperm quality of asthenozoospermic patients. The results obtained by this study have showed that silver NPs have improved sperm motility. Sperm motility was examined using simple layer technique (data not shown).

In recent years, extensive studies have emphasis the role of oxidative stress on causing male infertility. The excessiveaccumulation of ROS or RNS lead to oxidative stress, which in turn has harmful effect on sperm functionality [15-17]. Oxidative stress is a keystone cause of sperm dysfunction,since, it underline several mechanisms that lead to sperm dysfunction including; extensive sperm DNA damage, reduced sperm motility, decline in sperm fertilising ability, and defective sperm membrane integrity via lipid peroxidation[18-21]. In infertile male, major sources of ROS and RNS are produce by mitochondria in the spermatozoa and activated inflammatory leukocytes. In fact the activated leukocytes produce 1000 times more ROS/RNS than the spermatozoa does [1].

This study has estimated the concentration of SOD in sperm following their treatment with Doum extractwith/without silver NPs. Interestingly, the data of this study has showed for the first time the application of silver NPs have boosted the antioxidant effects of Doum extract by increasing SOD concentration. The results showed significant increase in SOD concentration in treated sperms in both Doum extract with NPs group and Doum extract only group compared with control group (****$p<0.0001$, **$p= 0.0032$ respectively) (Figure -1). Although, Doum extract with NPs group had slightly higher influence on SOD concentration when compared with Doum extract only group, but this was not significant ($p= 0.1229$).

Inside human body, oxidative stress occurs due to an imbalance occurs, causing potentially serious health consequences. The body has several defends scavenging mechanisms to maintain normal balance of ROS and RNS. These mechanisms include a number of antioxidant enzymes and antioxidant molecules. SOD provide a very important antioxidant defense mechanism against oxidative stress in the body.There is a strong evidence suggesting that foodstuff has a potential source of antioxidant phytochemicals, which act to prevent or reduce oxidative stress by scavenging free radicals , such as vitamins and, in particular, the flavonoids, coumarins, hydroxycinnamates and lignin component. Many studies have reported that Doum extracts contain significant amounts of flavonoids which have demonstrated antioxidant and antibacterial activities[3, 22, 23]. Flavonoids possess a significant biochemical property to act as antioxidant. The mechanisms of antioxidant action of flavonoids can include; scavenging ROS and/or

RNS, upregulating or protection of antioxidant enzymes defenses, or by inhibition of enzymes involved in free radical generation [24-26].

Spermatozoa are susceptible to ROS-induced damagedue to contain large quantities of polyunsaturated fatty acids. Also, it has been suggested that ROS induce membrane lipid peroxidation in sperm[27, 28]. Since,MDA is one of toxic lipid peroxides that known to cause different impairments of sperm cells,therefore, this study has examined the level of Lipid peroxidation formMDA.

Pathological high level of ROS, which may originated from endogenous sources or exogenous sources can be potentially toxic to sperm, leads to curtail sperm damage and impaired sperm functionality. In fact, sperm dysfunction occursdue to the peroxidation of high polyunsaturated fatty acids found within the plasma membrane of spermatozoa[29]. This study has measured the level of MDA(as an indicator of lipid peroxidation) in sperm samples following their treatment with Doum extract with/without silver NPs. The results showed that silver NPs have significantly enhanced the uptake of Doum extract and consequently the Doum extract has significantly reduced the level of MDA when compared with the non-treated group (*p= 0.045) (Figure -2). This finding demonstrated the significant role of the implication of silver NPs to amplify Doum extract effect. Also, the finding has emphasised the antioxidant influence of Doum extract, asan antioxidant has an important role in reducing lipid peroxidation.The implication of silver NPs has enhanced the therapeutic effect of Doum extract on reducing MDA.However, the underlying mechanisms of action still uncovered, therefore, further investigation to fully understand the role of silver NPs and medicinal properties of Doum extract is required. This study suggest that the implication of nanotechnology in ART to treat infertile male has a significant impact effect on enhancing sperm quality and increase the chances for pregnancy.

Moreover, oxidative stress has serious effect on the integrity of sperm DNA. One of sperm DNA damage causes,is the presence of unbalanced ROS in semen plasm. Sperm DNA damage is the main cause to infertility, miscarriage and birth defects in the offspring[30].

The data obtained by this study has showed significant therapeutic effect of Doum extract on improving and reducing sperm DNA fragmentation. Moreover, the results showed there was significant higher therapeutic effect on improving and reducing in the percentage of DNA-fragmented spermatozoa when silver NPs was implicated with Doum extract. In fact, both treatment groups (Doum extract only group or Doum extract with NPs group) were significantly enhanced the percentage of DNA-fragmented spermatozoa when compared with non-treated group i.e. control group ***p = 0.0002, **** p <0.0001 respectively (Figure 3).Interestingly, the data of this study has showed for the first time the application of silver NPs with Doum extract have remarkable and significant influence on decreasingthe percentage of DNA-fragmented spermatozoa compared with group treated with Doum extract only **p= 0.0033 (Figure -3). Research has been reported that oxidative stress had a great role in the aetiology of this pathological condition. Based on this finding, the reduction in the percentage of DNA-fragmented spermatozoa may ascribed to the antioxidant activity of Doum extract. Many studies have suggested that the aqueous extract of Doum fruits had an antioxidant activity; this was attributed to the substantial amount of their flavone components. About five flavone glycosides were isolated and identified from Doum fruits these include: viz, luteolin 7-O-β-glucuronoide, apigenin 7-O-β-glucuronoide, luteolin O-β-glycoside, luteolin 7-O-rutinoside and chrysoeriol 7-O rutinoside[22]. Clearly, there is a need for future studies that could address the fundamental molecular basis of mechanisms of action of biological activities of Doum extract.

Conclusion

The study has demonstrated for the first time the application of nanotechnology for ART to enhance sperm quality has a beneficial impact.The study has proved that enhancing the levels of antioxidant defense mechanisms could improve sperm functionality. Actually, the study has showed that the antioxidant therapeutic activity of Doum extract has improved the level of SOD, reduced MDA and reduced the percentage of DNA-fragmented spermatozoa. It is worthy to mention that this antioxidant activity was significantly higher when silver NPs was implicated with Doum extract. Although, there is still need to understand the mechanisms of action that underlying the biological and therapeutically activities of Doum extract components but,clearly, the findings in this present study has provided an additional set of information and encourage the application of nanoparticles with ART to bridge the gap between traditional uses of medicinal herbs and nanotechnology.

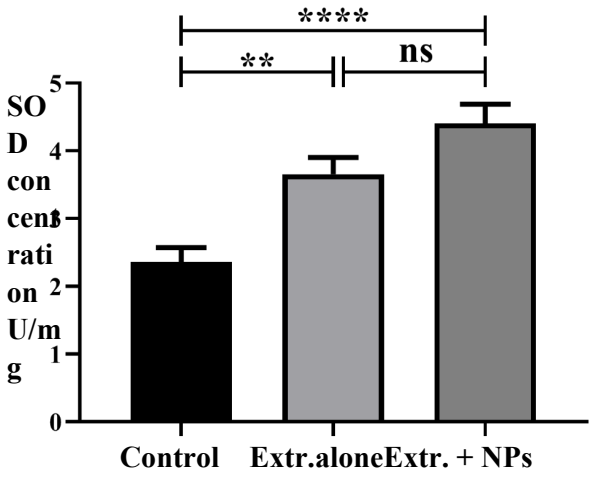

Figure 1: Silver NPs have significantly upgrade the antioxidant effect of Doum extract by elevating the level of SOD.

The level of SOD was measured using colorimetric assays. The differences between groups were tested for significance using Ordinary one-way ANOVA of One-way ANOVA with Sidak's multiple comparisons test. Data shown as M±SEM. *p* values of <0.05 were considered significant. (Extr. Alone= group were treated Doum extract only and Extr. +NPs = group were treated with Doum extract with silver NPs).

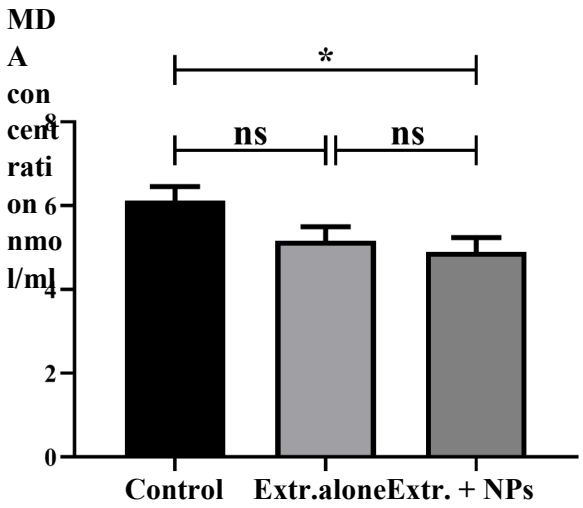

Figure 2: Silver NPs have significantly upgrade the antioxidant effect of Doum extract by reducing the level of MDA.

The concentration of MDA was measured using colorimetric assays. The differences between groups were tested for significance using Ordinary one-way ANOVA of One-way ANOVA with Sidak's multiple comparisons test. Data shown as M±SEM. *p* values of <0.05 were considered significant. (Extr. Alone= group were treated Doum extract only and Extr. +NPs =group were treated with Doum extract with silver NPs).

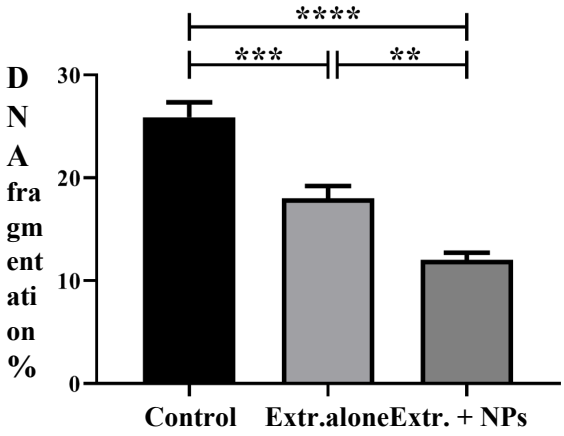

Figure 3: Silver NPs have significantly enhanced the effect of Doum extract on improving DNA fragmentation index.

The percentage of sperm DNA fragmentation was reduced when sperms were treated with Doum extract either with or without silver NPs. The differences between groups were tested for significance using Ordinary one-way ANOVA of One-way ANOVA with Sidak's multiple comparisons test. Data shown as M±SEM. *p* values of <0.05 were considered significant. (Extr. Alone= group were treated Doum extract only and Extr. +NPs =group were treated with Doum extract with silver NPs).

Conflict of Interest: No

Source of Findings: Self

Ethical Clearance: No

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Musculoskeletal Hand Manifestations in Patients with Diabetes Mellitus

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Abstract

A random sample of 505 patients with DM, evaluated in a discriptive study for the evidence of MSK hand manifestations of DM, they were collected attending diabetic centre, in Sulaimani city, from Novembor 2008 to February 2009 . All the patients were interviewed by using questionnaire forms . Comprehensive history, clinical examination, radiological, and laboratory studies performed to role out other causes of musculoskeletal hand manifestations . Chi- square and ANOVA procedure have used for management of statistical data. One hundred ninety six diabetic patients had MSK hand manifestations represents (39.52%), with nearly equal male and female involvement, majority of the patients with DM (84.2%) were type 2, while most of patients with hand manifestations (47.7%) were type 1 DM, (50.3%) of them had DM for less than 5 years, most of them (71.9%) use OHD for treatment of diabetes, and (51.1%) of them had uncontrolled glycemic control status. The relation of hand manifestations with gender, duration, type of treatment, and glycemic control status of DM was statistically significant (P value < 0.05) . Our patients got different hand manifestations with significant association with gender, duration, type of treatment and glycemic control status of DM . Majority of the patients with MSK hand manifestations had type 1 DM . MSK hand manifestations were more in patients with uncontrolled blood glucose, with almost equal male and female involvement .

***Key words**: Musculoskeletal hand; Diabetes mellitus; T2DM; MSK*

Introduction

Diabetes mellitus (DM) is a syndrome with disordered metabolism and inappropriate hyperglycemia due to either a deficiency of insulin secretion or to a combination of insulin resistance and inadequate insulin secretion to compensate. Diabetes mellitus is classified on the basis of the pathogenic process that leads to hyperglycemia, as opposed to earlier criteria such as age of onset or type of therapy [(1-3)]. The two broad categories of DM are designated type 1 and type 2. Both types of diabetes are preceded by a phase of abnormal glucose homeostasis as the pathogenic processes progresses. Type 1 diabetes is the result of complete or near-total insulin deficiency[(4-6)]. Type 2 DM is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased glucose production[(8-11)]. Diabetes mellitus is associated with a wide variety of musculoskeletal problems, some of which are unique to the disease [(7)] including Syndromes of limited joint mobility (Diabetic hand syndrome, Adhesive capsulitis, Trigger finger, Dupuytren's contractures), Osteoporosis, Diffuse idiopathic skeletal hyperostosis, Neuropathies (Charcot joints, diabetic osteoarthropathy [(12-14)], Carpal tunnel syndrome, Diabetic amyotrophy, Reflex sympathetic dystrophy,Various other neuropathies), and Diabetic muscle infarction [(15-17)] . As early as 1957, it was noticed that many people with diabetes complained of stiffness in the hands or stiff joints. This was eventually called limited joint mobility (LJM)[(18)] . LJM is also known as diabetic cheiroarthopathy (after the Greek word "cheiros" for hand)[(19)] or diabetic stiff hand syndrome[(14,15)] . In Dupuytren's contracture, a thickening and shortening of the palmar fascia occurs[(20-23)]. The aim of the current study was to find out the prevalence of MSK hand manifestations of DM to make early diagnosis, and to identify its relation with type, duration, and type of treatment of DM.

Materials and Method

Five hundred and five patients with DM were evaluated in this descriptive study The patients recruited consecutively with clinical diagnosis of DM by consultant physicians . They were attending diabetic centre in Sulaimani city, in a period of 4 months from Novembor 2008 to February 2009 .

Patients were interviewed, history taken, examination done for each case by using special questionnaire forms, patients with MSK hand manifestation sent to laboratory tested for (erythrocyte sedimentation rate, C reactive protein, rheumatoid factor, general urine examination, renal function test, liver function test) and conventional radiograph of hands to role out other causes of hand manifestations. The control status of DM assessed according to International Diabetes Federation (IDF)-2005 guidelines which considers that the patient is strictly glycemic controlled if HbA1c is < 6.5% or fasting blood sugar < 110 mg/dl or random blood sugar < 145 mg/dl (glycosylated hemoglobin HbA1c was not available, so we depend on fasting or random blood sugar).

Statistical analysis

Statistical package for social science (spss) program version 15 was used for statistical analysis, the frequency distributions were obtained, after the grouping of data to different variables.

Findings

The sample enrolled 505 patients with DM diagnosed by consultant physician, 175 (34.65%) patients were males and 330 (65.35%) were females (Figure 1), patient's ages ranged from 13 to 82 years with mean of ages 52.21 years and standard deviation 12.02 (Table 1) .

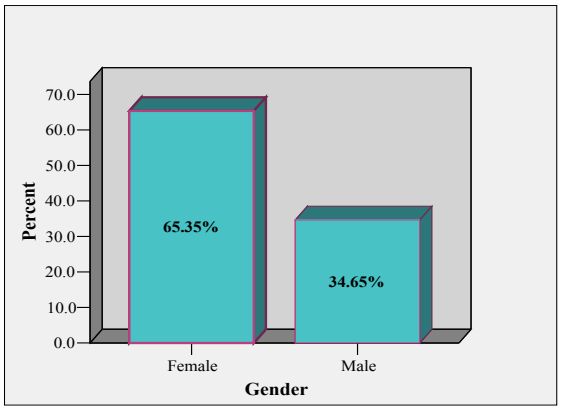

Figure 1: Sex distribution of DM

Table 1: mean age of diabetic patients

Variables	Minimum	Maximum	Mean	Std. Deviation
Age (Years)	13	82	52.21	12.02

Majority of diabetic patients was type 2, 452 (84.2%) . 363 (71.9%) of them were on oral hypoglycemic drugs and the others were on insulin, mixed therapy, or diet alone . About half of the patients 254 (50.3%) had diabetes mellitus (DM) for less than 5 years . According to the glycemic control status 247 (49.9%) were controlled while 258 (51.1%) were not . The family history of DM were almost equal, 243 (48.1%) had positive family history of DM, and 262 (51.9%) had negative history (Table 2) .

Table 2: Type, duration, treatment, control status, and family history of DM

Variables	**Frequencies**	**Percentages**
Type of DM		
Type 1	80	15.8
Type 2	425	84.2
Duration of DM		
Less than 5 years	254	50.3
6-10 years	118	23.4
11-15 years	59	11.7
16-20 years	38	7.5
More than 20 years	36	7.1
Treatment of DM		
Diet	23	4.6
Oral hypoglycemic drugs	363	71.9
Insulin	79	15.6
Mixed	40	7.9
DM control status		
Uncontrolled	258	51.1
Controlled	247	48.9
Family history of DM		
Negative	262	51.9
Positive	243	48.1

The MSK hand manifestations of DM are summarized in (Figure 4-2) which shows that 196 (39.52%) patients had MSK manifestations in the hand which were cheiroarthropathy, flexor tenosynovitis, Dupuytren's contracture, and CTS { 69 (13.91%), 48 (9.68%), 42 (8.47%), 37 (7.46%) } consecutively, while 309 (60.48%) patients had no MSK hand complications .

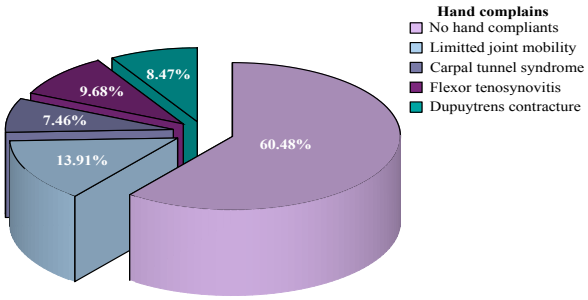

Figure 2: MSK hand manifestations of DM

In Table 3, we found a positive relation between MSK hand manifestations and duration of DM which is statistically highly significant (P value = 0.000), with a mean of (2.222 ± 1.304) showing that frequency of hand complications increase with duration of DM, while there is no relation with age (P value = 0.119) .

Table 3: Relation of MSK hand manifestations with age and duration of DM

Hand complains	Age		Duration of DM	
	Mean+ S.D	P value	Mean+ S.D	P value
No hand compliants Limited joint mobility Carpal tunnel syndrome Flexor tenosynovitis Dupuytrens contracture	51.46±12.01 53.25±12.54 49.76±10.88 53.44±11.45 55.76±12.73	0.119	1.74±1.10 2.04±1.29 2.16±1.40 2.50±1.29 2.67±1.44	**0.000**

Discussion

Diabetes mellitus (DM) is associated with a wide variety of musculoskeletal (MSK) problems, some of which are unique to the disease . The nomenclature of these conditions can be confusing, with some having more than one name in the medical literature. Out of 505 patients we found 196 (39.52%) had one or more types of MSK hand manifestations including (LJM in 13.91%, flexor tenosynovitis in 9.68%, Dupuytren's contracture in 8.47%, and CTS in 7.46%). This result is comparable to that of Sanjeeb *et al* [28] who recorded the LJM to be the most common MSK hand manifestations followed by flexor tenosynovitis, then Dupuytren's contracture, and lastly CTS . Sanjeeb *et al* mentioned the frequency for each hand manifestation but didn't mention the frequency of total number of MSK hand manifestations in his patients . In the other hand in a study done in Greece by Kim *et al* [29] found the prevalence of MSK hand manifestations as high as (82.6%) which is higher than ours.

In a recent study done in USA, Rachel P.K. et al.[11] claiming prevalence of LJM among diabetic patients to be (8 - 50%) which is very wide range, while in a study done by Grgic *et al* [30] the prevalence of LJM was (28.4%) . Triasman HS. et al.[31] reported the prevalence of LJM as (8.4%), while Das *et al* [32] reported it as high as (30-40%) . In Ardic *et al* [33] which is a study done in Turkey, found that non of his patients had LJM and this might be due to short duration of DM in patients he chose . The prevalence of flexor tenosynovitis in our study (9.68%) is lower than that of Mackenzie *et al* [34] in which the prevalence was (11-63%), and higher than that of Ardic F.et al.[33] who shows that (3.8%) of diabetic patients had flexor tenosynovitis . Rachel et al.[11], and Kozak *et al* [35] reported the frequency of Dupuytren's contracture continuant to be (16-42%) and (12-32%) consecutively, this figure having a wide range and higher than ours . Regarding the prevalence of CTS, it was (20%) in a study done by Rachel *et al* [11] which is higher than ours (7.46%), while it is only (1.3%) in that of Ardic *et al* [33] . The difference in the prevalence of MSK hand manifestations of DM may be belongs to the difference in the duration, type, and glycemic control status of DM between the patients included in each study . We found the relation of MSK hand manifestations with duration of DM is highly significant and shows that MSK hand manifestations increase with duration of DM and this is consistant with the results of Petrulewicz-Salamon *et al* [36], Enrico Cagliero *et al* [37], Aydeniz *et al* [38], Sanjeeb *et al* [28], Starkman *et al* [39], Rachel *et al* [11], and Yosipovitch [40] who all reported the MSK hand manifestations to be increased with duration of DM . Regarding gender the occurrence of MSK hand complications were had almost equal males and females with a significant statistical level in our

study, while other authors like Enrico Cagliero et al.[37] in a study done in USA recorded higher prevalence of hand complications in their female patients . A number of author looked at relation between type of DM and MSK hand complications . Douloumpakas *et al* [41] found that MSK hand complications is more in type 1 DM, while Petrulewicz-Salamon *et al* [36] found that it is more in type 2 DM, while in ours the relation of type of DM and MSK hand complications did not reach a significant level. Starkman *et al* [39] recorded that hand complications were more common among diabetic patients on insulin therapy. Glycemic control is being the key treatment of DM, according to our results there is a significant relation between MSK hand manifestations and glycemic control status (P value = 0.027) and shows that MSK hand manifestations were more common in patients with uncontrolled glycemic status .This result is comparable with that of Petrulewicz-Salamon I. et al.[36], a study done in turkey by Aydeniz *et al* [38], and a study done in India by Sanjeeb *et al* [28], while it is differ from that of Enrico Cagliero *et al* [37] which shows that there is no relation between glycemic state and hand manifestations .

Conclusion

Our patients got different hand manifestations with significant association with gender, duration, type of treatment and glycemic control status of DM . MSK hand manifestations were more in patients with uncontrolled blood glucose, with almost equal male and female involvement .

Conflict of Interest: non

Source of Findings: self findings.

Ethical Clearance: This research was carried out with the help of my colleagues mentioned with me. The oral and paper approval of the patients in this study, which included the withdrawal of blood samples from them and laboratory tests.

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Correlation Study between *H.pylori* Bacteria Qnd Biochemical Tests In Type 2 Of Iraqi Diabetic Patients

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Abstract

Background/Aims: many important pathogenic bacteria as Helicobacter pylori (H. pylori) can infect diabetic patients and causing inflammation, therefore aimed of the current study was study the Correlated between a demographic characteristic and biochemical tests of H.pylori in type 2 of Iraqi diabetic patients. **Materials and Methods:** study was carried out on diabetic patients infected or non-infected with *H. pylori*, during period (February-July)2017,in specialized centre for endocrinology and diabetes, study group (Patients) , collected information from all patients by pretested questionnaires , biochemistry testes were done by enzymatic methods. **Results:** Out of the 185 patients of type 2 diabetes, *H. pylori* infection was found higher prevalence of *H. Pylori* infection in diabetics (38.4%) as compared to non diabetic patients (59.4%), as well as significant increase FBG(228 ± 27.9) ; HbAlc(8.1± 1.2) ; *S.*Creatinine(1.3 ± 0.4); Cholesterol(303.7± 9.8), HDL(40.6± 33.2) and LDL (146.4± 26) levels in *H. pylori* seropositive diabetic patients whilst increased level of WBCs (7.1±2.3); HB(13. 23± 0.9); S.Albumin(5.3 ± 0.1) and triglycerides (164.9 ± 17.4) in *H. pylori* seropositive non-diabetic patients. **Conclusion :**higher prevalence of *H. Pylori* infection in diabetics more than no diabetic patients , also high rate of *H. pylori* infection among age group (35-45) years , and significant increase FBG; HbAlc; *S.*Creatinine ; Cholesterol, HDL and LDL levels in *H. pylori* seropositive whilst increased level of WBCs ; Hb; Albumin and triglycerides in *H. pylori* seropositive non-diabetic patients.

Keywords: *H. pylori (Helicobacter pylori*);biochemical tests ; type 2 diabetic patients.

Introduction

Helicobacter pylori are considered as worldwide public health problems which more prevalent in the developing countries, mainly acquired in childhood by the oral-oral; fecal-oral; or gastro-oral route [(1)] .so *Helicobacter pylori* (*H. pylori*)- induced gastritis could potentially affect the secretion of important hormones such as ghrelin; leptin ; somatostatin ; gastrin, and somatostatin, that could affect insulin sensitivity and glucose homeostasis, also, other mechanisms may be involved in the possible causative correlated between infected of *H. pylori* and T2DM[(2).]

In worldwide, About 3.8 million deaths patients have type 2 diabetes mellitus [(3)] as well as many previous showed high correlated between *H. pylori* infection and T2DM[(4)] .Many important factors are important in the development of H. pylori-associated gastroduodenal diseases, such as host factor (as sex; age as well as genetic susceptibility); virulence antigens and environment-related factors[(5)].

Materials and Method

The study was carried from (February 201– July) 2018, in specialized centre for Endocrinology and diabetes , Collected 5 ml of blood from diabetic patients infected or non-infected *H. pylori* and for clotting , left at room temperature for (1) hour , the centrifuged at 3000 rpm for 10 minutes and serum was at – 20C until the used.

Biochemical test : the biochemistry testes were done in morning after fasting overnight (12hours), collected venous blood sample for testing : fasting blood sugar (FBS) level ; glycosylated haemoglobin (HbAlc); Total cholesterol ; HDL (high-density lipoprotein

cholesterol; Triglyceride; serum creatinine and FBG measured for both study and control group by routine enzymatic methods , used the diagnostic kit (Biolabo SA.;France), All testing were done in the laboratory of specialized center for Endocrinology and diabetes in Baghdad. Diagnosis of *H.Pylori* by used ELISA (IgG, EIA , Trinity Biotech , U.S.A).

data analysis:

Values were expressed as mean ±SD, differences between the mean values were analyzed by chi- square test ,the criterion for significance was ($p \leq 0.05$) .

Finding :

In table 1 appearance prevalence of *H.pylori* infections in diabetic patients were (15.6%) in the age group(35 45) years;(10.2%) in (25 – 35) years whilst in control group (non- diabetic patients) was 13% and 21.7 %, respectively which was founded significant ($P = 0.5$), and showed high percentage in age group (≥ 45) years , so in this table the gender high distribution in male (24.4 , 20.5)% in both groups (diabetic patients and healthy) compare to female (14, 14.7) % with and without diabetes mellitus .

Table (1): distribution of diabetic patients and Control Group(healthy) according to *H.pylori* infected .

Type Groups	*H.pylori*				Total (No.,%)
	(+)		(-)		
	No.	%	No.	%	
Study Group (diabetic patients)	35		57		92
Control Group	50		34		84

The present study found higher prevalence of *H. Pylori* infection in diabetics (38.4%) as compared to nondiabetic (59.4%) .

Table (2): Corelationshipe between biochemical test and *H.pylori* in diabetic and non- diabetic patients .

Biochemical test	Study group (diabetic patients No.= 185)		Control group (healthy voluntary No.= 185)		Normal value (mg/ dl)
	H.pylori		*H.pylori*		
	(+)	(-)	(+)	(-)	
F.B.G	228 ± 27.9	203.4 ± 22	95.0 ± 12.11	89.9± 12.3	60 -110
W.B.Cs	6.9 ± 1.5	6.5 ± 1.4	7.1±2.3	6.9 ±2.1	4.0 – 11.0
Hb	10.2± 0.7	10.6 ± 0.8	13. 23± 0.9	12.50± 0.12	
HbA_1c	8.1± 1.2	7.9 ± 0.7	4.96±0.43	4.1±0.43	3.0 – 6.0
S. Albumin	4.5 ± 0.5	4.2± 0.5	5.3 ± 0.1	4.3 ± 0.5	3.5 – 5.3
S. Creatinine	1.3 ± 0.4	0.9 ± 0.3	0.50 ± 0.08	0.61 ± 0.05	0.2 - 1.2
S.Triglycerides	151.9± 3.9	106.7± 13.2	164.9 ± 17.4	130.4± 3.2	< 150
Cholesterol	303.7± 9.8	236.1± 16.5	201.3 ± 5.2	209.7± 5.5	130- 200
HDL	40.6± 33.2	41 ± 18.8	42.9 ± 3.2	40.5 ± 3.0	< 40 male < 50 female
LDL	146.4± 26	134.2± 16.8	133.5 ± 20.3	135.6±21.0	< 100

The (mean values± S.D) of FBG ; WBCs ; HB ; HbAlc ; S.Albumin ; S.Creatinine ; triglycerides ; Cholesterol, HDL and LDL for diabetic patients type2 infected with H. pylori(+) and non infected(-) compare to control were illustrated in table(2).

The present results showed significant increase FBG(228 ± 27.9) ; HbAlc(8.1± 1.2) ; *S.*creatinine(1.3 ± 0.4); Cholesterol(303.7± 9.8), HDL(40.6± 33.2) and LDL (146.4± 26) levels in *H. pylori* seropositive diabetic patients whilst increased level of WBCs (7.1±2.3); HB(13. 23± 0.9); S.Albumin(5.3 ± 0.1) and triglycerides (164.9 ± 17.4) in *H. pylori* seropositive non-diabetic patients.

Chen and Blaser showed that H.*pylori* play directly or indirectly in increases HBA_{lc} levels in the adults[11], so Chen and Blaser ,2012 elucidate *H. pylori* regulates leptin and ghrelin, which play important role in energy homeostasis and metabolism[11], so several studies as [12,13] showed elevation or increased level of LDL in *H. pylori* infection, so [14] found in *H. pylori*-infected patients have higher serum cholesterol and lower HDL than in negative patients ,However regarding HDL, similar results were previously reported[15, 16, 17].

Aarabi*et al.,* 2010 appearance in his study level of triglyceride level was higher in *H. pylori* infection than in negatives[12]. However VolanenI *et al.,*2005found that serum triglyceride and HDL level can change during acute phase of bacterial infection[18].

Glycated haemoglobin (HbAlc) was correlated to glucose levels measurements obtaining that HbAlc could be used as an objective tool of glycemic control[19] other hands [7]elucidate increased levels of HbAlc associated with *H. pylori* infection in type 2 diabetes group[7] , As well as HbAlc in *H. pylori* infection among diabetics were greater than *H. Pylori* non diabetics[7] ,but [20] founded no correlated between *H. pylori* infection with HbAlc in men aged40-70 years with or without diabetes , Whilst [21] found that *H. pylori* eradication in type 1 diabetes does not affect glycemic control in patients as evidenced by HbAlc level and daily insulin requirement.

So Bajaj *etal*., 2014 study provides evidences that *H. Pylori* infection was associated with increase in LDL level and triglyceride but no effect on levels of both total cholesterol and HDL[7].

In healthy Korean adults was associated with lipid profile as increase in LDL ; total cholesterol and triglyceride, but decrease in HDL[22] .

Dursen *et al*., found no significant in lipid profile in both *H. pylori* infected in diabetics and non-diabetics[23,24].

Conclusions

H.pylori infections are one of the risk factor may be considered in the evaluation of diabetic patients .

H.pylori infections were higher in diabetic patients compare to healthy controls.

Serum cholesterol, FBG; HbAlc; *S.* Creatinine ; Cholesterol, HDL and LDL were higher level in *H. pylori*-positive diabetic group as compared to negative diabetics.

incidence of bacteria in smokers, and who drinking the coffee; tea and water also source of water (natural or minerals) in both groups (diabetic and non-diabetic), and highly prevalent in low income patients.

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Conflict of Interest : None

Source of Funding : Self

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

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Correlation of Serum Amylin Level to Polycystic Ovarian Syndrome

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Abstract

Background: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age, affecting 6%–10% of women worldwide. Objective: The study aimed to assess the relation of serum amylin level in women with polycystic ovarian syndrome. **Materials and Methods:** A cross−sectional study was carried out in Salah Al-din City from 10th of November 2018 , to 20thof May 2019. The number of polycystic ovary syndrome women under study was 51 women whose ages were between 18 and 45 years old. These women admitted to obstetrics and gynecology unit at Salah Al-din Teaching Hospital. The control group who were matched to the patients studied included 37 women.F**inding :**The study showed that there is the significant difference between PCOS women and the control group concerning serum amylin level and the highest mean of amylin was occurred in PCOS women (183.12 ± 10.021 vs. 23.62 ± 1.97). There was a weak positive correlation between body mass index and amylin level among PCOS women in this study. The highest rate of PCOS women had irregular menstrual cycle. The study showed that most PCOS women included in the study had hirsutism. The high rate of acne recorded among PCOS women. **Conclusion:** It was concluded that there was a highly significant relation of amylin to PCOS.

Keywords: *Hirsutism, menstrual irregularity, polycystic ovary syndrome, amylin.*

Introduction

Polycystic ovary syndrome is the most common endocrine disorder in women of reproductive age, affecting 6%–10% of women worldwide.(1) Polycystic ovary syndrome is characterized by chronic anovulation, hyperandrogenism, and multiple small subscapular cystic follicles in the ovary on ultrasonography.(2)

Amylin is a 37 amino acids polypeptide hormone with a molecular weight of 3.9 kDa, was firstly isolated from amyloid deposit in pancreatic islets of type 2 diabetic patients.(3) Amylin is mainly co-secreted with insulin by pancreatic β-cells in response to stimulation of glucose, free fatty acids, and nutrient intake.(4) Amylin inhibits food intake and delays gastric emptying, leading to the reduction of blood glucose levels and body weight. Moreover, pramlintide, an amylin analogue also reduces body weight in humans.(5) However, studies assessing serum amylin levels in PCOS women have yielded inconsistent results(6).

Materials and Method

A cross-sectional study was carried out in Tikrit City from 10th of November 2018 to 20th of May 2019. The number of PCOS women under study was 51 women whose ages were between 18 and 45 years. The diagnosis of PCOS was based on Rotterdam Criteria: oligo and/or anovulation clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries in ultrasound, meaning presence of 12 or more follicles measuring 2 – 9 mm in diameter in each ovary and/or ovarian volume more than 10 cm^3. In addition the control group consisted of 37 apparently healthy volunteer females with regular menstrual cycles aged between 18 and 45 years. A volume of 5 mls 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, the samples were centrifuged at 3000 rpm for 15 min then if a clot was developed, then was removed and re-centrifuged at 3000 for 10 min, and the obtained serum were aspirated using mechanical micropipette and transferred into clean plain tubes with

screw which labeled and stored in deep freeze at - 20 °C for the biochemical measurement of amylin by ELIZA.

Statistical Analysis

The statistical analysis was performed using Statistical Package for the Social Sciences version 23 (SPSS, IBM Company, Chicago, USA).

Finding

This study showed that there was a significant difference between PCOS women and the control group concerning serum amylin level. The mean serum amylin in PCOS women was 183.12 ± 10.021 vs. 23.62 ± 1.97 in control group, as shown in table 1. There was a weak positive correlations between body mass index (BMI) and serum amylin level among PCOS women in this study, figure 1. The highest rate of PCOS women had an irregular menstrual cycle (84.31%) and 15.69% of them had normal menstrual cycle, as shown in figure 2. The study showed that most PCOS women included in the study had hirsutism (66.70%), while only 33.30% were without hirsutism, as shown in figure 3. The high rate of acne recorded among PCOS women was 53% and 47% of PCOS women without acne, as shown in figure 4.

Table (1) : Serum level of amylin in women with PCOS and the control group.

Amylin level (pg/ml)	PCOS women	Control group	t. test	P. value
No.	51	37		
Mean	183.12	23.62	7.28	0.0001
SD	10.021	1.97		

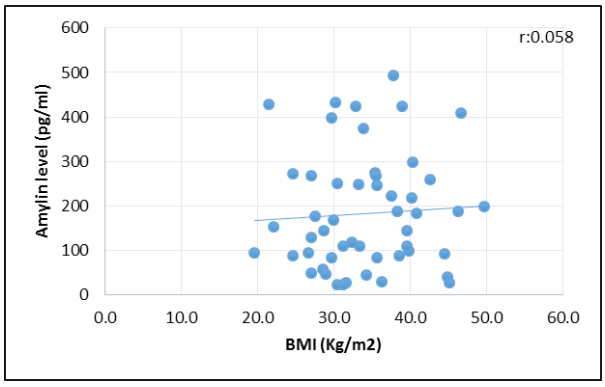

Figure (1): Correlation between amylin hormone and BMI of PCOS women.

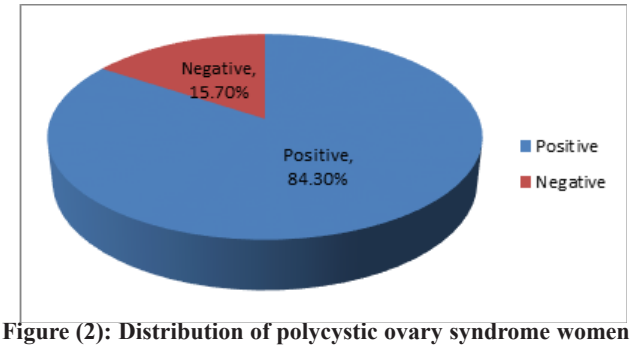

Figure (2): Distribution of polycystic ovary syndrome women according to irregularity of menstrual cycle.

Figure (3): Distribution of PCOS women according to hirsutism.

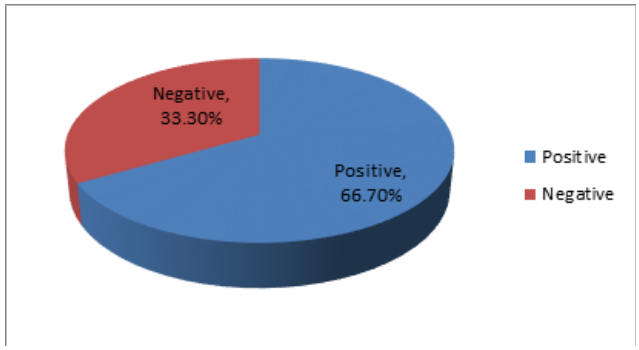

Figure (4): Distribution of PCOS women according to presence of acne.

Discussion

PCOS is an endocrine disorder with multifactorial etiology and various clinical manifestations. It is the most common cause of menstrual disorder and an ovulatory infertility in women.[7]This study showed that there is the significant difference between PCOS women and the control group concerning amylin level which was found to be significantly higher in women with PCOS comparing with the apparently healthy women at of less than 0.01, suggesting the presence of a hormonal disturbance in women with PCOS, which may reflecting a significant effect of the pancreatic hormones on the ovarian function. These results were in agreement with JAMES *et al,* [8] who found an increment in serum amylin level in women with PCOS. The precise mechanism of increased serum amylin levels in women with PCOS currently is unknown, PCOS is a complex disorder and carries an important pathophysiological role. Therefor women with PCOS have a higher chance for insulin resistance and compensatory hyperinsulinemia leading to an increased risk of obesity, type-II diabetes mellitus, metabolic syndrome, as well as hyperandrogenemia. Amylin may contribute to their progression of diabetes, and beyond their risk for diabetes, women with PCOS also have higher risk of developing dyslipidemia and

hypertension.[9]

This study showed that the rate of PCOS women to had an irregular menstrual cycle, hirsutism, and acne were higher in women with PCOS as compared with the control group.some studies report a prevalence of hirsutism in women with PCOS in the range of 50–76%. [10][11] Hirsuitism is defined as excessive hair growth in area usually associated with male sexual maturity, that is, on the face, chest, lineal alba, lower back, buttocks, and anterior thighs. Hirsuitism results from androgen effects on the pilosebaceous unit and is commonly associated with acne and oily skin. It is usually due to an increase in androgen production from the ovaries or adrenal glands.[12] The results noticed by this study were in agreement with Azziz *et al* [13] and Ovalle and Azziz, [14] they concluded the source of hyperandrogenism due to the genetic abnormalities in insulin receptor resulting in the thickening of the ovarian theca that increased the androgen production and inhibition of sex hormone binding globulin synthesis. The degree of hirsutism might be influenced by the relative activity of the 5α-reductase enzyme that convert testosterone to the more active metabolic dihydrotestosterone.[15] [16] The high concentrations of testosterone, one of the factors that contribute to some symptoms of PCOS such as infertility, polycystic ovaries, hirsutism, and acne.[17] Gowri *et al* [18] showed that the acne was seen in highest percentage (67.5%), followed by hirsutism (62.5%) in women with PCOS compared to healthy women of a comparable age group and fasting insulin levels was the most common hormonal abnormality seen along with both acne and hirsutism. Sharma *et al* [19] Majumdar and Singh [20] also showed that acne was the most common cutaneous manifestation in PCOS group.

In addition, women with PCOS are frequently obese and obesity is associated with menstrual cycle abnormalities in the general population.[21] The prevalence of these cycle abnormalities may vary in unselected women with PCOS because they reflect the subgroup of PCOS women who have sought medical attention and thus are more likely to demonstrate a more severe phenotype.[22] On the other hand, menstrual cycle irregularity is a relatively accurate surrogate of ovulation and is easily obtained from the medical history.[23] *Strowitzki et al* [24] reported that in women with PCOS, insulin resistance was significantly lead to an amenorrhea (cycle length > 6 mo) when compared with those with normal cycles.

Conclusion

In conclusion, women with PCOS had a higher serum level of amylin compared to other women without PCOS of a similar age group.

Conflict of Interest: Non

Source of Findings: Self

Ethical Clearance: Nil

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MMP-13 Gene Polymorphisms Associated with KOA in Iraqi Population

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Abstract

Knee osteoarthritis (KOA) is disease occur in knee joint characterize by cartilage degradation that mediate via pro-inflammatory marker such as matrix metalloproteinase-13 (MMP-13) . MMP-13 is enzyme expression in chondrocyte ,encoded by MMP-13 gene , act to degraded it and joint degenerative .This study explained association the rs2252070 single nucleotide polymorphisms (SNP) of MMP-13 gene in KOA . The result demonstrate that G alleles have significant association with KOA development . our results are the first ones reporting an association between MMP-13 and KOA in Iraqi populations .

Key words */ MMP-13 gene polymorphisms and Knee osteoarthritis (KOA).*

Introduction

Knee osteoarthritis (KOA) is the disease occur in the knee joint .It's degenerative arthritis in cartilage matrix of knee , is a group of overlapping distinct diseases, which may have different etiologies but with similar at biologic, morphologic, and clinical outcomes[(1)]. The most common form of arthritis is KOA , 6%of adults are affected it and the women more affected than men at age more than 45 years .KOA always has many soluble mediators for example prostaglandins and cytokines ,these mediators can stimulat matrix metalloproteinase-13 (MMP-13) in chondrocyte. MMP-13 production is consider the first step in theory of inflammation and degenerative knee joint[(2)] .

MMP-13 (also called collagenase) is enzymes that break the collagen peptide bonds in cartilage (Collagen, a key component of the extracellular matrix of chondrocyte) encoded by MMP-13 gene. MMP-13 is a protein of 81 875 Da and 739 amino acid residues as deduced from the DNA sequence analysis [(3)] . MMP-13 protein is synthesized in precursor form (preprocollagenase), then the signal peptide and pro region are removed and the resulting mature form is secreted. Plays a role in the degradation of extracellular matrix proteins(cartilage degradation) including fibrillar collagen and fibronectin .Cleaves triple helical collagens, including type I, type II and type III collagen, but has the highest activity with soluble type II collagen [(4)] .

Materials and Method

Study design : This study designed according to approved via Institutional Ethics Committee in Medical College / Al-Nahrain University / Iraq and it done in Rheumatology & Rehabilitation Consultation Unit in Al-Yarmouk Teaching Hospital / Iraq . The consents obtained from all patients and healthy persons that involved in the study . Diagnosis of cases with KOA was according to American College of Rheumatology (ACR) , all the patients are classified as primary KOA will include in this study .The ACR depends on signs and symptoms of KOA that show on patients also depend on clinical examination and X- ray (anteroposterior (AP) view and skyline view) [(5)] . This study involved collected 50 primary KOA grade 1 (G1) and 50 grade 4 (G4) cases and 50 controls, age of the cases and controls were more than 40 years . Table 1.

Table 1 : Numbers and age of the KOA cases and controls

	Number	**Age (years)**
KOA (G1) cases	50	Mean ±SD (48.1± 5.8)
KOA (G4) cases	50	Mean ±SD (48.8± 6.1)
Controls	50	Mean ±SD (49.1± 6.6)

SD/ Standard deviation

Samples collection : Five ml of blood drawed from cases and controls collected it in EDTA tube .DNA was isolated from blood sample by DNA extraction kit (Zymo company / USA) according to special method at kit . The isolate DNA stored in -20 C until future use . The DNA sample had purity ratio 1.7 to 2 that used in PCR-RFLP method . The Gene Runner software (Version 3.05) was used to design of MMP-13 gene primer , all primer information in table 2.

Table 2: Data for analysis of SNP in the human MMP-13 gene.

Gene name	SNP	Primers	GC %	Restriction enzyme	Tm (C)	PCR product size (bp)
MMP-13	rs2252070	Forward 5'- GAT ACG TTC TTA CAG AAG GC- 3'	45	BsrI	50.4	445
		Reverse 5'-GAC AAA TCA TCT TCA TCA CC - 3'	40		48.7	

PCR used for DNA amplification : Polymerase Chain Reaction (PCR)The extracted used to amplification of DNA . PCR was applied on AURA TM PCR Cabinet (Labnet – Italy). The PCR reaction was consisted of 1.5µl of 110 ng DNA, 1μL of mM dNTPs , 10 pmols/µl (1µl) of forward and 10 pmols/µl(1µl) of reverse primer , 0.3 μL of 5U/µl iTaq DNA polymerase , 3 μL of Taq polymerase Buffer A , and 16.5 µl of deionized water . The thermal cycling conditions showed in table 3.

Table 3: The thermal cycling conditions

No.	Phase	Tm (^{o}C)	Time	No. of cycle
1-	Initial Denaturation	95^{o}C	3 min.	1 cycle
2-	Denaturation -2	95^{o}C	45sec	35 cycle
3-	Annealing	56^{o}C	45sec	
4-	Extension-1	72^{o}C	45sec	
5-	Extension -2	72^{o}C	7min.	1 cycle

The production of PCR were separated by use a 2% agarose gel containing 2 μL ethidium bromide. A 100 bp DNA marker was used as a size standard for each gel lane. The UV light used for visualization of gel

Restricted Fragment Length Polymorphism (RFLP) Analysis: The productions of PCR was digested by restriction enzyme , BsrI (Biolab/England). The digested fragments were electrophoresis on 2.5% agarose gel mixed with red stain. The genotype frequencies. found by RFLP analysis .

Statistical Analysis

The GraphPad PRISMstatistical software (version6 .0, San Diego, CA, USA) was used to data analyses . The Chi-square (χ 2) test used to find the difference between allelic frequencies and genotype in KOA cases and control. The odds ratio and confidence interval (CI) (95%) was used to calculate association between KOA cases and control's genotypic and allele frequencies . Odds ratio was used to statistically demonstrate a positive dose response relationship of mutant allels (G allele) with KOA .

Finding

A / KOA Patients: This study involved 50 patients with G1 KOA and 50 patients with G4 KOA ,and

studied Genotype frequencies by using PCR -RFLP to detect the found of SNP of MMP gene (rs2252070).

rs2802292 SNP is characterized by A>G in G1 KOA patients .The AA mutant type (homozygous) of genotype frequency was high (58.7%) , and GG mutant type was low (2.1%) also AG mutant type (heterozygous) was (39.1%) . This study calculated the allel frequencies by genotype frequencies and showed that A allele was high (79%) but G was low (21%) in G1 KOA patients .

rs2802292 SNP is characterized by A>G in G4 KOA patients .The AA mutant type (homozygous) of genotype frequency was (26%) , and GG mutant type was low (20%) also AG mutant type (heterozygous) was high (54%) . This study calculated the allel frequencies by genotype frequencies and showed that A allele was high (57%) but G was low (43 %) in G4 KOA patients .

B / Controls. This study involved 50 healthy persons and studied Genotype frequencies by using PCR -RFLP to detect the found of SNP of MMP gene (rs2252070) .

rs2802292 SNP is characterized by A>G in controls .The AA mutant type (homozygous) of genotype frequency was high (56%) , and GG mutant type was low (0%) also AG mutant type (heterozygous) was (44%) . This study calculated the allel frequencies by genotype frequencies and showed that A allele was high (78%) but G was low (22%) in controls .

C/ Association Studies : This study included compared between G1 KOA with controls and G4 KOA with controls . The results showed that χ 2 forG1 with controls were non significant but for G4 with controls were significant correlation. The odds ratio and Cl 95% forG1 with controls were non significant but for G4 with controls were significant correlation . As per genotype frequency analyzed data, KOA cases are more likely to have a AG allele as compared to AA and more likely to have a GG allele. These association depend on CI that consider significant when to be above 1.0. If GG allele can be considered as a higher exposure to G as compared to AG , then the above statistics demonstrate a positive dose-response relationship of G allele with KOA . Table 4, 5and 6 .

Table 4: Comparsation of control with G1according to MMP13 alleles polymorphisms

Polymorphism	Group								X^2	Sig.	P-value	CI 95%	OR	df
	Control N=50				G1 N=50									
	Observed	Percentage	Expected	Percentage	Observed	Percentage	Expected	Percentage						
AA	28	56	22.36	44.72	28	58.7	22.36	43.83	0.155	NS	0.6934	0.631 to 1.356	0.848	1
AG	22	44	26.9	53.8	20	39.13	25.80	52.74						
GG	0	0	0.74	1.48	2	2.174	1.90	3.435						
Total	50	100	50	100	50	100	50	100						
X^2	3.055				4.149									
Significant	NS				NS									
P-value	0.2171				0.1256									
Allele frequency														
A	0.78				0.79									
G	0.22				0.21									

Table 5: Comparsation of control with G4according to MMP13 alleles polymorphisms

Polymorphism	Group								X^2	Sig.	P-value	CI 95%	OR	df
	Control N=50				G4 N=50									
	Observed	Percentage	Expected	Percentage	Observed	Percentage	Expected	Percentage						
AA	**28**	**56**	**22.36**	**44.72**	**13**	**26**	**19.81**	**48.11**						
AG	22	44	26.9	53.8	27	54	21.7	**47.36**	**6.123**	*	**0.0133**	**1.091 to 2.197**	**3.253**	**1**
GG	0	0	0.74	1.48	10	20	8.5	4.6						
Total	50	100	50	100	50	100	50	100						
X^2	3.055				9.519									
Significant	NS				**									
P-value	0.2171				0.0086									
Allele frequency														
A	0.78				0.57									
G	0.22				0.43									

Table 6: Comparsation of G1 with G4according to MMP13 alleles polymorphisms

Polymorphism	Group								X^2	Sig.	P-value	CI 95%	OR	df
	G1 N=50				G4 N=50									
	Observed	Percentage	Expected	Percentage	Observed	Percentage	Expected	Percentage						
AA	**28**	**56**	**22.36**	**43.83**	**13**	**26**	**19.81**	**48.11**						
AG	**20**	**40**	**25.80**	**52.74**	**27**	**54**	**21.70**	**47.36**	**7.633**	**	**0.0057**	**1.152 to 2.534**	**3.833**	**1**
GG	**2**	**4**	**1.90**	**3.435**	**10**	**20**	**8.5**	**4.6**						
Total	**50**	**100**	**50**	**100**	**50**	**100**	**50**	**100**						
X^2	**4.149**				**9.519**									
Significant	**NS**				**									
P-value	**0.1256**				**0.0086**									
Allele frequency														
A	**0.79**				**0.57**									
G	**0.21**				**0.43**									

Discursion

The KOA is degenerative disease of cartilage in knee joint , it's inflammatory disease because it occur with inflammatory mediators and the age consider as risk factor to OA development therefore consider as within age related diseases [1].

The chondryocyte with age suffer of high expression of nitric oxide (NO) (high level of NO act as risk factor against survival of cell) this considered as factor for chondryocyte degredation and OA development due to the NO is one of triggers for MMP-13 in cell [6]. MMP-13 are enzymes that break the peptide bonds in collagen (Collagen, a key component of the animal extracellular matrix, is made through cleavage of pro-collagen by collagenase once it has been secreted from the cell) encoded by MMP-13 gene and it's act as mediator the cartilage degradation [7].

This study showed association between MMP-13 gene polymorphisms with KOA through the mutant of A>G . This association explained when compare control with G4 KOA and G1 with G4 KOA and obtained significant association in this comparisons. This result demonstrate that MMP-13 gene polymorphisms can develop the KOA , This effect occur by NO that mediate the MMP-13 expression.

NO act as 2nd messenger , such as reactive oxygen species , in cell for activation of many biological functions therefore it can activate the Guanylate cyclase (GC) . GC act to convert the GTP to cGMP .cGMP mediate the phosphorlation of protein kinase G (PKG) and activation it (8). PKG also act to phosphorlate the Core-binding factor alpha 1 (Cbfa1) in presence of cGMP exclusively ,this lead to activation of Cbfa-1 . Cbfa-1 is localize in cell nuclei , this lead to it effect on MMP-13 gene and elevate MMP-13 expression [9] . Therefore , the NO cGMP- PKG pathway promot MMP-13 expression .The NO cGMP- PKG pathway might drive Cbfa-1-dependent gene expression in chondryocte by regulating nuclear translocation of Cbfa-1. This can be stimulate and hyperactivation of proinflammatory cytokines (such as MMP-13) [10].

Our study for the first time shows that MMP-13 SNP (rs2252070) is significantly associated with KOA in Iraqi population . In addition to the highly significant association of MMP-13 and OA , based stratification also indicated that the mutant "G" allele has a much more pronounced risk rate of OA in Iraqi populations . Increase of G allele have association with elevate MMP-13 activity and KOA development . The our study is first time shows association between KOA and MMP-13 SNP (rs2252070) , and some study in China (Sun G1, Ba CL1 and et al . 2019) show same association but different allele , at Chinese population the A allele but in Iraqi G allel is risk factor in KOA development due to it can increase the encoded and activity of MMP-13 [11].

Conclusion

The our study conclude that genotype frequencies of KOA patients and controls were studied using PCR-RFLP technique to detect the presence of SNP of MMP-13 gene (rs2252070). The Chi-square test indicated significant positive relationship between KOA and TG and GG genotypes

Conflict of Interest: Non

Source of Findings: Self

Ethical Clearance: Non

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Rejuvenation of Under Eye by Nanofat Grafting

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Abstract

Over the past decade, we have seen unprecedented growth in the popularity of elective cosmetic surgery. In a prospective study, a total number of 22 female patients under the age of 40 years (range from 20-40 years) all complaining form dark pigmentation involving the lower eyelids in Kirkuk city in north of Iraq ,all patient had been treated by intradermal nanofat injection . Those patients were selected, evaluated and operated during the period from March to November 2018. In all cases the diagnosis confirmed clinically; none of these patients in this study received previous medical or surgical therapy. Their ages were ranging between (20 years to 40 years). All were treated by single stage nanofat injection in the lower eyelids ;Nanofat injected to improve the pigmentation and skin rejuvenation for all patients injected with intradermal nanofat ; all patients injected by nanofat using small sharp needle 27-gauge in repeated fan-shaped patterns intradermally with 1 cc of nanofat in each side followed by Simple digital massage over the lower eyelids ;at the end of the injections erythema and a yellowish discoloration was noticed in whole lower eyelids; this discoloration disappeared within 5 weeks by a gradual lightening of the skin. After an average follow-up of six months (range 3–9 months), Assessment of the results by the surgeon were good in 50%, fair 36.4 and poor 13.6, patients satisfaction were good in 59.1%, fair in 31.8% of cases and only 9.1 were poor as shown in. Only 2 patients (9.1%) in this study had prolong ecchymosis as postoperative complications , all other patient healing pass smoothly and uneventful .

Keyword: *Lower Eyelid Skin; Nanofat grafting; fat graft*

Introduction

Over the previous decade, unprecedented growth in the popularity of elective cosmetic surgery have been seen. According to the American Society for Aesthetic Plastic Surgery, there has been a approximately 8% elevation in plastic surgery[(1)]. different factors can cause dark circles around the eyes. One cause is excessive pigmentation due to dermal melanocytosis. This can be congenital or occur secondary to such conditions as atopic or allergic contact dermatitis [(2)]. Another common cause of dark circle is a thin, translucent lower eyelid skin overlying the orbicularis oculi muscle. Transparent eyelid skin allows visualization of the underlying subcutaneous vascular plexus or vasculature within the muscle [(3)]. Shadowing due to skin laxity and tear trough is another cause of infraorbital dark circle [(4)]. This can worsen with time because of age-related loss of subcutaneous fat and hollowness of the periorbital area. Many causative factors of the dark circles include translucent, thin skin with excessive pigmentation of the lower eyelids skin overlying the orbicularis oculi muscle and shadowing due to laxity of the skin and tear trough deformity, periorbital edema, superficial location of vasculature may also exacerbate the condition [(5)]. Etiologic factors likewise incorporate hereditary/ established, post provocative hyperpigmentation ,dermal melanin affidavit, hypersensitive contact dermatitis, optional to sickliness, stress, flawed propensities [(5)]. So this condition may be challenging to treat due to it complex etiopathogenesis, and lacking straightforward and repeatable therapeutic options [(6)]. Many treatment modality had been used like IPL, and Q-switched ruby laser, local creams ,Chemical peels, as well as fillers have all been tried for treatment but none have provided a satisfactory treatment [(7)]. All mentioned treatment option are not effective for patients with dark pigmentation in lower lid due to thin, translucent skin. In this type of dark pigmentation, injection of autologous fat has been reported as a good method [(8)]. A new treatment technique called Nanofat has been introduced by Tonnard *et al*. In which autologous fat graft harvested manually

by closed syringe lipoaspiration then it's emulsified mechanically until a liquid suspension is obtained and then superficially injected in intradermal plain with fine sharp needles [2]. Autologous fat grafting firstly reports in the early twentieth century and used clinically after the introduction of liposuction by Illouz in the 1980s as a lipofilling treatment in which fat injected deep subcutaneously [9]. Zuke *et al* determined that stem cells could be isolated from human adipose tissue obtained by suction-assisted lipectomy (liposuction) was processed to obtain a fibroblast-like stem cells [10]. In this clinical study, nanofat technique evaluated the reliability of a practical method in which intradermal nanofat injection used to rejuvenate skin and treat dark pigmentation in lower eyelids.

Material and method

2.1. Patients:

In a prospective study, a total number of 22 female patients under the age of 40years(rang from 20-40 years) all complaining form dark pigmentation involving the lower eyelids in Kirkuk city in north of Iraq ,all patient had been treated by intradermal nanofat injection. Those patients were selected, evaluated and operated during the period from March to November 2018. In all cases the diagnosis confirmed clinically; none of these patients in this study received previous medical or surgical therapy. Their ages were ranging between (20 years to 40 years).

To evaluate clinically the benefit of Nanofat injection for skin rejuvenation and dark pigmentation of the lower eyelids as an outpatient procedures with short term follow up. Preoperative evaluation in all 22 patients included medical history, a complete physical examination, thorough history and physical examination. Collected information about patient associated illnesses, smoking, drug use and medication history, and family history, preoperative photograph were taken to all patients in frontal view. Liposuction was performed and harvested from the lower abdomen after infiltration with a modified Klein solution, prepared by 1 liter of physiological solution (RL) + lidocaine 800 mg with adrenaline 1mg (1:1000).

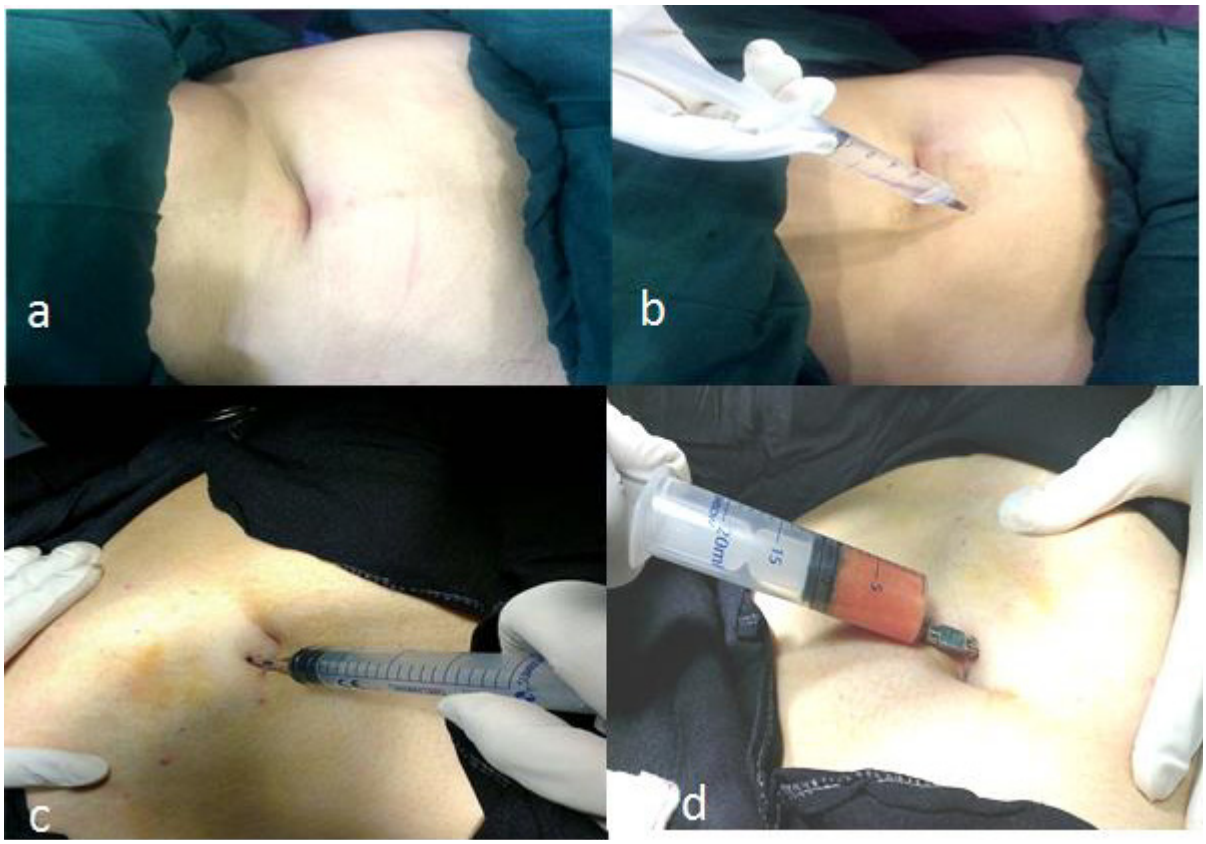

Fig 1. Operative procedure: (a)-Lower abdomen draping (b-c)- infiltration with a modified Klein solution (d)-Closed syringe liposuction.

Then the collected fat (Fig.2), in order to reduce any residual local anesthetic solution and red blood cells lipoaspirated fat was rinsed with sterile saline (Fig.2:2b), then by using Tonnard's technique [8] the lipoaspirate fat is mechanically emulsified this achieved by 30 passes shifting between two 10-cc syringes connected each other by a female-to-female Luer-Lock connector.

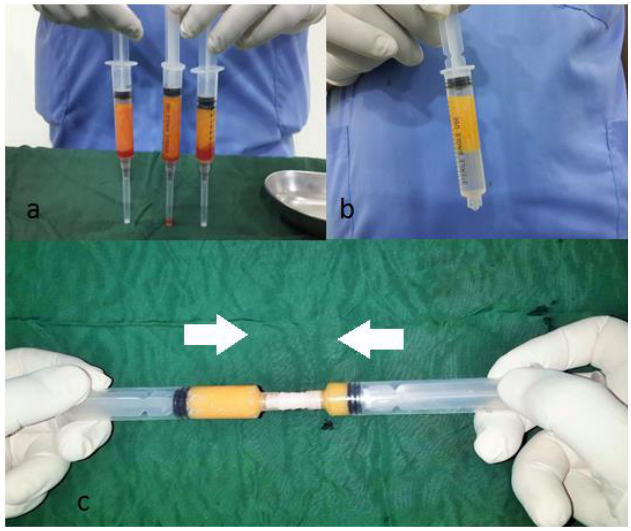

Fig 2: Nanofat preparation: (a) - Collected fat (b) - lipoaspirated fat was rinsed with sterile saline (c) - Emulsification of lipoaspirate fat by achieved mechanically by 30 passes shifting between two 10-cc syringes connected each other by a female-to-female Luer-Lock connector

Emulsification process liquefy fat to emulsion and changed it color from yellow to pale yellow white (Fig. 3b), the fat becomes liquid and emulsified so it can be injected easily by small needle 27-gauge intradermally (Fig. 3a) the injection started from medial aspect of the lower eyelids to lateral aspect (Fig. 3c-d) followed by simple digital massage to injected area (Fig. 3e) to prevent skin irregularity due to nanofat localization and also decrease post injection erythema and yellow discoloration

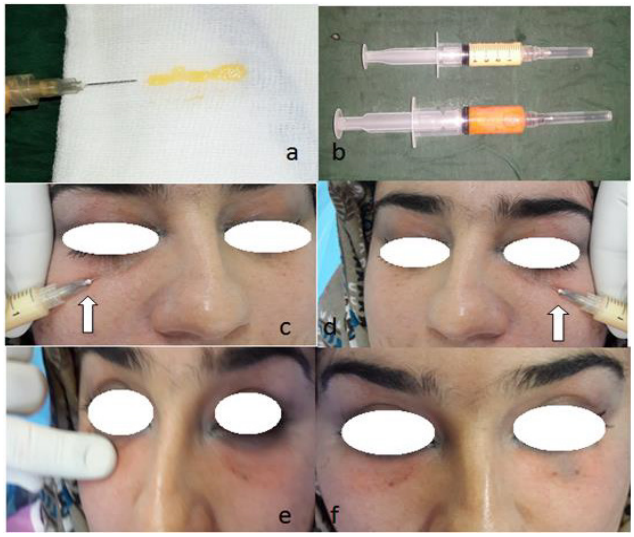

Fig 3 (a-f) Nanofat injection(a)- Nanofat infused effectively by little needle 27-measure (b)- Emulsification procedure modify fat shading from yellow to light yellow white (c-d)- Intradermal nanofat infusion in both left and right lower covers (e)- Simple computerized back rub to the infused region (f)- post infusion erythema and yellow staining.

2:5 Post-operative measure:

Dressing made with local eye ointment apply to the lower eyelids. Oral Antibiotics with local eye antibiotic ointment were continued for five days postoperatively, usually patients were return to normal life style on the 3th postoperative day. The patients were followed up (3 to 9 months post operatively) for the evaluation.

Findings

Twenty two female patients were included in this study ,there age range from 20 to 40 years as shown Table 1, Patients concern was variable between dark pigmentation alone or pigmentation with rejuvenation as shown in Figure 4; all were treated by single stage nanofat injection in the lower eyelids ;Nanofat injected to improve the pigmentation and skin rejuvenation for all patients who injected with intradermal nanofat ; all patients injected by nanofat using small sharp needle 27-gauge in repeated fan-shaped patterns intradermally with 1 cc of nanofat in each side followed by Simple digital massage over the lower eyelids ;at the end of the injections erythema and a yellowish discoloration was noticed in whole lower eyelids; this discoloration disappeared within 5 weeks by a gradual lightening of the skin. After an average follow-up of six months (range 3–9 months), Assessment of the results by the surgeon were good in 50%, fair 36.4 and poor 13.6, as shown in Table 2; patients satisfaction were good in 70%, fair in

25% of cases and only 5 were poor as shown in Table 3. There were no significant wound complications, no infections, no cysts, or permanent discolorations, or other side effects were observed right after the treatment and also at the follow up visit. ; Only 2 patients (9.1%) in this study had prolong ecchymosis as postoperative complications , all other patient healing pass smoothly and uneventful .

Table 1: Number and percentage of patients according to their ages

Age of patient	No. of Cases	Percentage
20-30 year	8	40
30-40 years	14	60

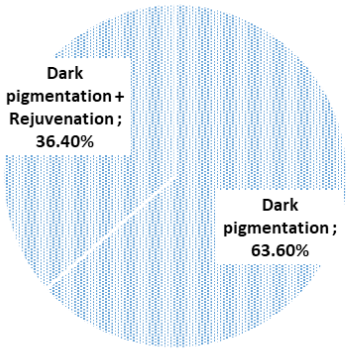

Figure 4: Rate of patients concern.

Assessment of the results by the surgeon as shown in table 3.3:

Table 2: Number and percent of surgeon assessment.

Result	No. of Cases	Percentage
Good	11	50 %
Fair	8	36.4%
Poor	3	13.6%

Table 3: Number and percent of Patients satisfaction.

Result	No. of Cases	Percentage
Good	14	70
Fair	5	25
Poor	1	5

Discussion

Dark circles under the orbital affect individuals of both sexes, all races, and a wide range of ages. Circumcision factors are known to cause the appearance of dark circles, including excessive pigmentation of the skin, thin, dry, thin skin that rises above the circular eye muscle, and shingles caused by skin laxity and tear pelvis. The most appropriate method of treatment varies according to the main causative factor of the dark circle. Topical skin lightening agents are the mainstay of treatment for hyperpigmentation [10]. Factors commonly used in hyperpigmentation include hydroquinone, retinoic acid, meicinol, azylic acid and corticosteroids [11]. Various combination therapies of these topical agents have been used to increase their efficacy while reducing side effects [12]. Autologous fat injection has been reported as an effective method for treatment dark lower lids pigmentation caused by thin, translucent skin in which many modalities of treatment cannot treat it [13]. But fat injection may cause visible lumps of fat, contour irregularities, or fat necrosis or fat loss as it injected subcutaneously [14]. Tonnard's *et al* change fat particles to semi liquid emulsify by mechanical emulsification process change fat particles to nanofat which completely disrupts the adipose tissue structure so it can be injected by small gauge needle intradermal not subcutaneously in order not to have a volume-adding effect. But the major benefit of nanofat injection is to relate to stem cell activity [8]. In the current study, nanofat grafting combined with microfat grafting produced satisfactory results in treating lower eyelid dark circles. Microfat grafting at the nasojugal folds compensated for the lack of volumetric effects of the nanofat grafting technique, and nanofat grafting at the lower eyelids improved the dark circles effectively, without any noticeable irregularity. This technique produces effects that are similar to those achieved by injecting purified stromal vascular fraction, but without the complicated technique or expensive laboratory equipment required for the latter. As well, the skin rejuvenating effects from stem cell activity are augmented by the fat graft acting as a soft tissue filler, to produce the final effect. In this study we have use nanofat injection by the same steps used by Tonnard's *et al*. in which they inject in each lower lids 1.6 cc of nanofat intradermally ;instead we use to inject 1 cc of nanofat in each lower eyelids and use simple digital massage to decrease postoperative erythema and pale yellowish skin discoloration which last for around

five week in compare to Tonnard's et al they notice that skin yellowish skin discoloration remained for 1 month postoperatively and eyelid skin erythema for three months after the procedure. In this study the final results was satisfactory, In all patients, healing was uneventful with no significant complications. The postoperative outcomes were satisfactory after an average follow-up of six months assessment of the results by the surgeon were accepted in more than 80% of all of the patients satisfaction also accepted in more than 80% of all the cases (good in 59.1%, fair in 31.8% of cases and only 9.1) and nearly the same results were obtained by other study. Most patients showed Satisfaction ranging from fair to good ,improvement in skin texture the lighting of dark skin color in the lower eyelids and this agreed with both Dong et al and Tonnard's *et al* which shows also good improvement and rejuvenation of skin and lighting of dark pigmentation; postoperative complications was minimal ,neither fat lumps nor texture irregularities were notice [14] and Tonnard's *et al* [8] also observed no significant complications. Intradermal Nanofat injection shows both rejuvenating affect and skin lighting due its Capability of stem cell production rather than volume build as in fat injection which injected in subcutaneous plain. As Tonnard's *et al* shows in there study that a number of good quality mesenchymal stem cells are still present in the nanofat sample [8].

Conclusion

our nanofat grafting technique provided substantial improvements.. Nano fat injection is one of the new modality of treatment which may consider an affordable option as an outpatient procedures that can be performed with ease in clinics to relive deep dark pigmentation and skin rejuvenation of the lower eyelids. This simple, cost effective procedure seems to be suitable for the correction of dark circles

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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