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Contents

SODIUM NITROPRUSSID AND ADENOSINE-ACTIVATED POTASSIUM CHANNEL IN AORTIC SMOOTH MUSCLE ISOLATED FROM FEMALE RATS

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Abstract:

Sodium Nitroprusside (SNP) and Adenosine (Ado) are potent drugs used in the treatment of cardiovascular diseases. Nitric oxid (NO) is produced from virtually all cell types composing the cardiovascular and regulates vascular function through fine regulation of excitation–contraction coupling. Adinosine endogenous metabolites play a major role in coronary autoregulation. Therefore, the aim of the present study was to investigate the contribution of NO and Ado mediated relaxation in rat aortic smooth muscle in intact and denuded endothelium rings precontracted with phenylepherine (PE). The thoracic aorta was isolated, cut into rings, and mounted in organ-bath chambers and isometric tension was recorded using powerLab Data Acquisition System (Model ML 870). According to the results of the current study, incubation of aortic rings with Glybenclamide (GLIB) decreased the relaxation response induced by Ado (the vasodilation value rate decrease from 41.07±6.7 control to 18.54±4.6) in intact aortic rings. L-nitroarginine methylester (L-NAME), not abolished the response induced by SNP, whereas Nifedipine significantly enhanced the response induced by SNP in a dose-dependent manner in intact endothelium rings. The relaxation to Ado in intact aortic rings was slightly decreased (6.88± 1.01), but not abolished completely after incubation with Caffeine (Ado receptors antagonist). On the other hand, removing endothelium did not attenuated the vasorelaxation induced by SNP and increased relaxation response. While, vasorelaxation of Ado in aortic rings were partially attenuated by removing endothelium. These results suggested that (1) ATP-dependent potassium channel (K_{ATP}) did not involve in SNP inducing vasorelaxation, while have a role in Ado mediated vasorelation. (2) Vasorelaxation effect of NO is endothelium independent, while, Ado relaxation effect is endothelium dependent.

Keywords: *Nitric oxide,Adenosine, Potassium channels, Aorta.*

INTRODUCTION

Nitric oxide (NO) and adenosine (Ado) mediates multiple physiological and pathophysiological processes in cardiovascular system (Ignarro *et al*., 2002). NO is known as a primary determinant of blood vessel tone and thrombogenicity, however the modulatory effects of NO on contractile function are undoubtedly complex (Massion *et al*., 2003). NO donor causes an increase in cyclic Guanosine monophosphate (cGMP) concentration, cGMP in turn stimulates Protein Kinas G (PKG) (Pfitzer 2001; Lincoln *et al*., 2001). PKG, elicits relaxation in vascular smooth muscle cells through a myriad of signaling pathways, leading to decreased intracellular calcium ion concentration $[Ca^{2+}]_i$ and desensitization of the contractile apparatus to Ca^{2+} (Carvajal *et al*., 2000). However, evidences exist for PKG-dependent activation of large-conductance Ca^{2+}-activated K^+ (K_{Ca}) channels and associated membrane hyperpolarization, inhibition of L-type voltage-gated Ca^{2+} channels, stimulation of Ca^{2+}-ATPases in both the plasma membrane and sarcoplasmic reticulum and inhibition of inositol trisphosphate receptors (Lincoln *et al*., 2001). Furthermore, NO can promote vascular relaxation through cGMP-independent mechanisms of smooth muscle relaxation by nitrosylation of cysteine thiol groups to post-translationally modify enzymatic activity (Resta, 2003). Lin *et al*., 2007 have been suggested that L-NAME, a competetive NO inhibitor, inhibit NO release.

Endogenous metabolites have been postulated to play a major role in coronary autoregulation (Makujina *et al*., 1994). The vasodilator action of Ado has generally been ascribed to stimulation of the A_2 Ado receptor subtype, probably acting by activation of adenylyl cyclase (AC) leading to elevation of cyclic adenosine monophosphate (cAMP) levels (Dart and Standen, 1993). However, it is unclear how A_{2A} receptor modulates the vascular response. In the guinea pig, A_{2A} Ado receptor is involved in coronary vessel relaxation, whereas A_{2B} receptor is present predominately in the aorta. Similarly, the vascular effects of adenosine in aorta and coronary vessel of rat have been reported to be mediated by both A_{2A} and A_{2B} receptors (Ponnoth *et al*., 2009).

The signal transduction pathway between Ado receptors and K_{ATP} channels in VSM is still

unknown. K_{ATP} channels in smooth muscle from mesenteric arteries and gallbladder can be activated through stimulation of cAMP-dependent protein kinase A (PKA) (Quayle *et al*., 1994).

The current study was designed to evaluate the contribution of potassium channel to SNP and Ado mediated relaxation in precontracted descending thoracic aorta. Furthermore, to find out the role of endothelium on NO and Ado mediated aortic relaxation.

MATERIALS AND METHODS

Animals

Female Albino rats (200-270 gm in weight) were used in the present study. Animals were housed in the animal house of Biology Dept., Faculty of Science, Zakho of University. Animals were kept at 22±2 °C and exposed to a regular diurnal cycles of 12-hours photoperid using an automated light-switching devise and had free access to water and food ad *libitum*.

Tissue preparation

The animals were injected intrapretoneally with heparin (2000 units/ 200 gm) and left for few minutes to avoid blood clotting and damaging of aortic endothelium. The Animals were anesthetized by placing them in a small cage and allowing them to inhale Diethyl ether (Deveci, 2006). Then, the descending thoracic aortae was carefully isolated and transferred immediately to Kreb's bicarbonate buffer solution with glucose and EDTA-to prevent the oxidation of unstable substances. The aorta was cleaned of periadventitial tissue and cut transversally into ring segments (each of 3 mm in length).

Measurement of vascular reactivity in isolated rat aorta

Each aortic ring was placed in a tissue bath filled with Kreb's buffer (37 ^{o}C), bubbled with carbogen (95% O_2 and 5% CO_2), and attached to a force transducer (Model FORT100) and connected to a PowerLab data acquisition system (Model ML845, ADInstruments, Australia). Computer running Chart software (version 7.0) was used for the measurement of isometric tension. Rings were allowed to equilibrate for 60–90 min at a resting tension of 2 g, before the addition of the blockers. The aortic segments were initially exposed to 60 mM K^+ to test their functional integrity. Later, the bath medium was changed several times until a resting tone was restored.

To test the role of K_{ATP} channels in the development of relaxation, the aortic rings were pre-incubated for 30 minutes with the 10 µmol/l Glibenclamide (K_{ATP} inhibitor) and Caffeine (3×10^{-4}mM). To test the effect of blocking NO synthetase in the presence of SNP, the aortic rings were preincubated with L-NAME. To investigate the effect of Nefedepine on SNP inducing vasorelaxation, the rings were pretreated with (10^{-5}mM) Nefedepine. Endothelial injury was induced by gentle rubbing of the intimal surface of the rings with a piece of PE 90 tubing and checked by the addition of Acetylcholine. Denuded endothelium rings were treated with SNP and Ado. Each ring was then contracted with PE (1×10^{-6} mM). Once a stable contraction was reached, cumulative concentration-response curves were obtained for SNP and Ado (1×10^{-7} to 3×10^{-4} mM).

Statistical analysis

All the data were expressed as means ± SEM. The median effective concentrations (IC_{50}) are given as geometric mean with 95% confidence intervals (CI). For comparison between means of two groups, two way ANOVA was used. P-values less than 0.05 were considered as statistically significant. All the graphs, calculation and statistical analyses were performed using GraphPad Prism software version 5 (GraphPad Software, USA).

RESULTS

Effect of GLIB on SNP and Ado Inducing Vasodilation

The involvement of K_{ATP} channel in inducing vasodilation to SNP and Ado was examined pharmacologically. A specific K_{ATP} channel blocker GLIB (1×10^{-5}M) was administrated to vessels to block the K_{ATP} channel activity. The results showed that the relaxation of the aorta to different concentrations of SNP pretreated with GLIB disinhibited and reduced vasoconstriction induced by PE in which the relaxation effect was reduced by 2.1±0.7.

The data of the current study showed the abolishing of the relaxation response potentiated by Ado in the presence of extracellular GLIB in PE (10^{-6}M) precontracted rats aorta and relaxation response was decreased from 41.07±6.7 control to 18.54±4.6 .

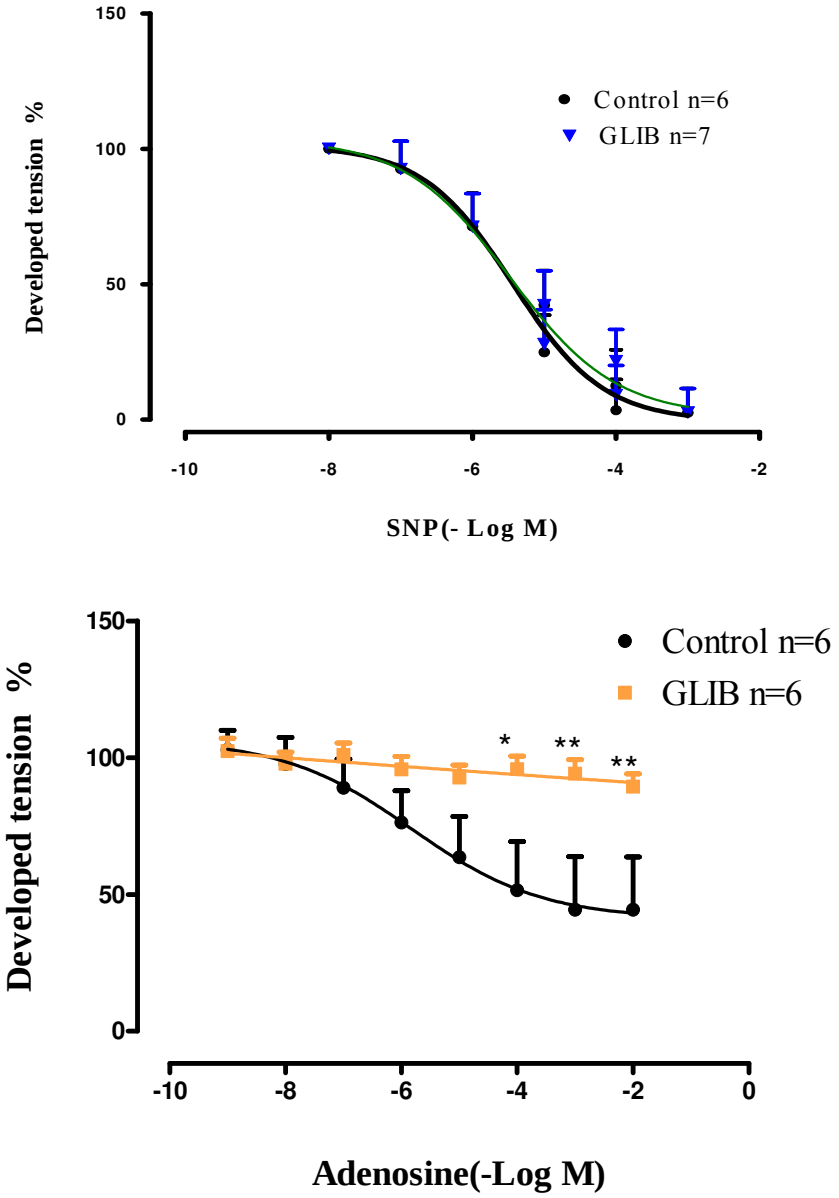

Fig 1. The effect of GLIB on the relaxation response induced by SNP and Ado in PE precontracted rat aortic rings. (A and B) Dose-response curve to SNP and Ado induced relaxation in control and in preincubated aortic rings with GLIB**.**

Effect of L-NAME on SNP Inducing Vasodilation

To investigate whether L-NAME as NOS antagonist have ability to abolish vasorelaxation induced in response to different SNP concentrations, we treat intact endothelium aortic rings with (3×10^{-4}M) L-NAME. As in previous experiments different concentrations of SNP were added to the aortic rings precontracted with PE (10^{-6}M) and preincubated with L-NAME in organ bath experiments. Vasodilation that produced in response to SNP in presence of L-NAME was decreased slightly but not abolished and vasodilation rate decreased by about 10.14 ±0.05.

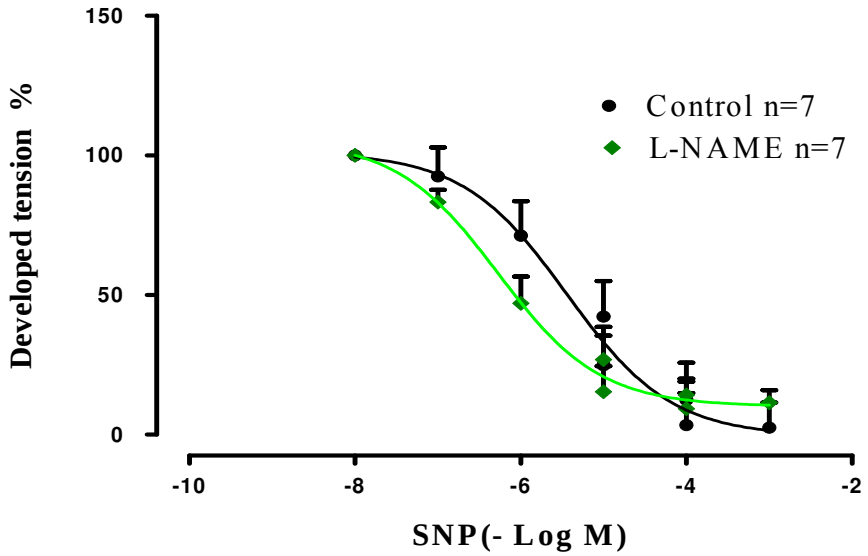

Fig 2. The effect of L-NAME on the relaxation reponse to SNP in PE precontracetd rat aortic rings. Dose-response curve to SNP and GTN induced relaxation in control and in preincubated rings with L-NAME.

Effect of Nifedipine on SNP Induced Relaxation

Nifedipine caused more rapid relaxation in aortic rings at low concentration of SNP (3×10^{-7}M), and the vasodilation produced was increased in comparatively by about 6.43±1.9 with Log IC50 -5.770.

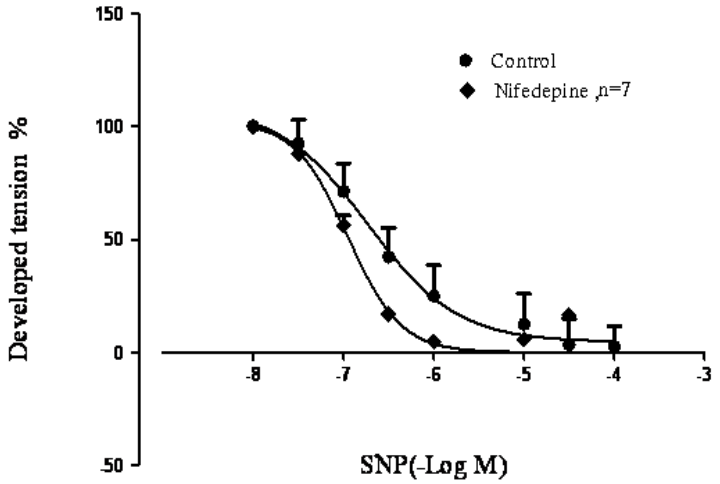

Fig 3. The effects of Nefedipene on the relaxant response to SNP in PE precontracted rat aortic rings. Dose response curve to SNP induced relaxation in control and in preincubated aortic rings with Nefedepine.

Role of Endothelium in SNP and Ado Inducing Vasorelaxation

To assess the role of endothelium in producing the vasorelaxation, the endothelium was initially removed to eliminate its contribution to vasodilation. Denuded endothelium aortic vessels were treated with different concentrations of SNP. The results showed that the relaxation produced by SNP is not affected by removing endothelium and the relaxation rate was reduced only by 3.3±1.1 with a Log IC50 -5.683. On the other hand, results showed that the disruption of endothelium attenuated Ado - induced vasodilation, and the relaxation was comparatively decreased from 47.37% in the control to 10.37%, with Log IC50 -5.105, -5.245 respectively, and the response of Ado relaxation at 3×10^{-3} mM was significant ($P<0.05$).

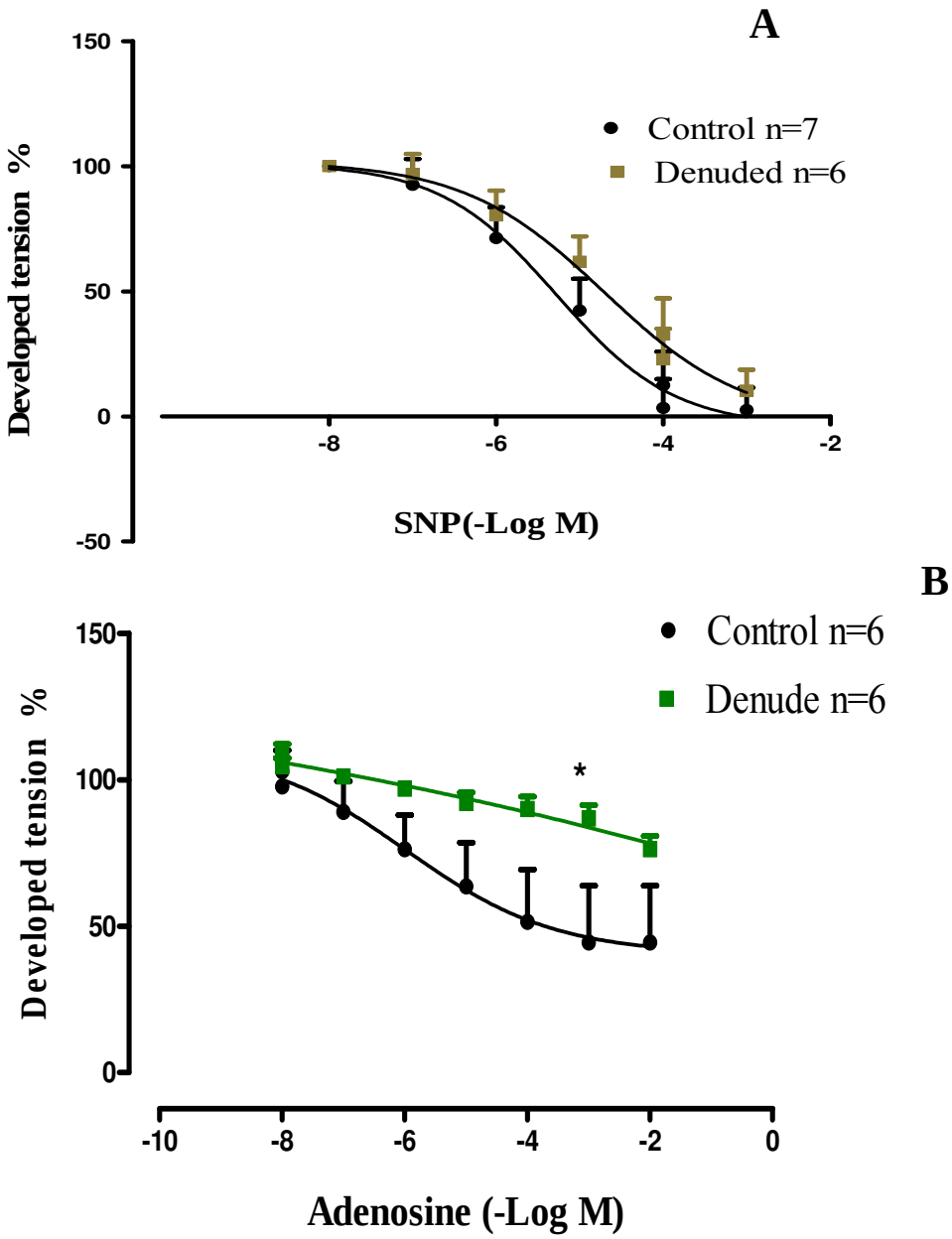

Fig 6. The effects of endothelium on the relaxant responses to SNP and Ado in PE precontracted rat aortic rings. (A and B). Dose response curve to SNP and Ado induced relaxation in control and in denuded endothelium aortic rings.

Discussion

In the present study, the mechanism of relaxation induced by SNP and Ado was further studied. To assess the role of K_{ATP} channel in SNP and Ado in inducing vasodilation, PE precontracted aortic rings were preincubated with GLIB showed disinhibiting vasodilation induced by SNP. This these indicate that SNP did not involved in opening of K_{ATP} channels to induce vasodilation.

To inhibit the synthesis of NO from L-Arginine in the presence of oxygen and by the help of NOS enzyme, L-NAME was used as NOS blocker. The current study showed that the NOS inhibitor effect of L-NAME did not attenuated or abolished SNP -induced vasorelaxation in rat's aortic smooth muscle. However the maximum relaxation for both SNP in presence of L-NAME slightly decreased as compared to control. This response may be due to direct dissociation of NO donor to produce NO without the action of NOS and also disexistance of exogenous precursor for NO. Therefore, any SNP induced changes in arteriolar diameter in the presence of L-NAME can be attributed to mechanisms independent on internal NO synthesis.

Nifedipine was used to test the role of NO blocking of calcium channel. The data of the current study showed that SNP significantly enhanced relaxation in smooth muscle. This is may be due to Ca^{2+} sensitization and the release of Ca^{2+} from one type of channel might increase the open probability of the other channel (Mayer et *al*., 2000 and Porter *et al*., 1998).

Active hyperporalization in rats aorta occurs when the release of NO from the endothelium is ongoing, so we examined the role of endothelium-derived NO in denuded endothelium rings induced vasorelaxation in the aorta using NO donors (SNP). Inhibition of the vasorelaxation induced by NO donors in rings with damaged endothelium not observed in the present study. This is may be due to direct dissociation of NO donor when added to solution without requiring to be released from endothelium. In the current study, our results indicated that K_{ATP} channels were not involved in SNP inducing relaxation in aorta with intact endothelium, while, the dilation response of aorta to Ado was attenuated by endothelial removal, suggesting that endothelial Adenosine receptors contribute in Ado vasorelaxation.

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کورتی:

سودیوم نایتروبروسایت (SNP) و ئەدینوسین (Ado) کاردکەن وەك دەرمانێن چالاك ل چارەسەرکرنا نەخوشین دلی. نایتریك ئوکساید (NO) دهێتە بەرهەم هێنان ل زوربەی خانێن دل و لوولەی و کاردکەن بۆ ڕێکخستنا کارێ لوولەی ب ڕێکخستنەکا هویر بۆ پروسێسا هاندان و کرژبونێ.Ado کو رولەکێ سەرەکی یێ هەی ل ڕێك و پێك کرنا شاخوێنبەری. ژ بەر هندێ ئارمانجا ڤێ ڤەکولینێ دیارکرنا بەشدارکرنا NO و Ado ل خاوکرنا ماسولکێن لوس ل شاخوێنبەری ب خانێن داپوشەری و بێ داپوشەرێ هاتینە جوداکرن ژ چوردێن سپی کو بەری هنگی هاتینە کرژکردن ب ڤینایلفرین.

شاخوێنبەری ٍسینك هاتینە چوداکرن ژ چوردی و پارچەکریە بۆ پارچێن بازنەیی و هێلایە دناف (organ-bath chambers) و ژمارتنا شداندێ هاتیە تومارکردن بکارئینانا Power Labو Acquisition System Model ML Data 870)

و لدیف ئەنجامێن ڤێ ڤەکولینێ , GLIB خاوکرنا د پارچێن شادەماری ئەوا دروست بوی ژ لایێ Ado کێم کر (نرخێ خاوبۆنێ کێم کر ژ ٤١,٠٧ ± ٦,٧ کونترول بۆ ١٨,٥٤±٤,٦ ل پارچێن شادەمارێ هاتیە داینکر بGLIB .ئەف کیمکرنە هاتە بەروڤاژی کرن ل بەرسفا SNP بۆ خاوکرنا لووڵی پشتی شادەمار هاتیە داینکرن ب LNAM ی و بەرسفا SNP بۆ خاوبونی نەگرت، ژ لایێ دیڤە، نیفدپین ب شێوەیەکی باش خاوبون زێدەترکر ل شادەمارێهاتینە چالاك کرن ب چرێن جوراوجورێن SNPی. خاوبون بۆ Ado هاتیە کێمکرن نێزیکی (٦,٨٨± ١,٠١) پشتی داینکرنا پارچێن شادەمارێ داپوشەر ب کافاینێ, بس ب تمامی نە هاتە گرتن. لابرنا داپوشەری ژی بروسێسا خاوبونێ کێم نەبو بەروڤاژی بروسێسا خاوبونێ بلندکر.

الملخص:

نايتروبروسايد الصوديوم (SNP) والادينوساين (Ado) ادوية القوية يستخدم في علاج أمراض القلب والشرايين. ويتم إنتاج النيتريك كسيد (NO) تقريبا من جميع أنواع الخلايا الذين تتكون منهم القلب والأوعية الدموية وينظم وظيفة الأوعية الدموية من خلال التنظيم عملية الاستثارة و التقلص. Ado المادة الأيضية التي تلعب دورا رئيسيا في تنظيم ذاتي للشريان التاجي، ولذلك، كان الهدف من هذه الدراسة التحقيق في مساهمة NO و Ado في الاسترخاء حلقات العضلات الملساء الأبهر سليمة والمعرية البطانة المعزلة من الجردان, وتم استحداث تقلص باستخدام مادة phenylepherine (PE). تم عزل الشريان ابهر ، وقطع إلى حلقات، وربط في الجهاز organ bath. تم تسجيل شدة العضلة باستخدام powerLab 870 Model ML) Data Acquisition System).

وفقا لنتائج هذه الدراسة، انخفضت استجابة الاسترخاء الناجم عن Ado في حلقات الأبهر المحضنة مع Glybenclamide (GLIB) (معدل انخفاض قيمة توسع الأوعية ٤١,٠٧± ٦,٧ في Control و١٨,٥٤ ± ٤,٦ في حلقات الأبهر المحضنة مع GLIB). L-nitroarginine methylester (L-NAME), لم تلغ الارخاء الناجم عن SNP، في ناحية أخرى نيفيديبين ادت الى زيادة كبيرة في الارخاء الناجم عن SNP بطريقة تعتمد على الجرعة في حلقات سليمة البطانة. كافاين قلت الاسترخاء الناجم من (Ado) في حلقات الأبهر سليمة البطانة ولكن لم تلغ تماما (٦,٨٨ ± ١,٠١) مقارنة مع الكنترول. من ناحية أخرى، إزالة البطانة في حلقات الأبهرية لم يخفف عملية الارخاء الناجم عن SNP. بينما، قلت جزئيا الارخاء الناجم من Ado في حلقات الأبهر العارية من البطانة. هذه النتائج اضهرت أن (١) قناة البوتاسيوم معتمد علىATP لم تشارك في ارتخاء وعائي الناجم من SNP ، في حين يكون له دور في vasorelation بوساطة Ado. (٢) ارتخاء الناجم من NO لا يعتمد على البطانة، في حين، بطانة لها تاثير جزئى على Ado.

THE PROTECTIVE ROLE OF CERTAIN ANTIOXIDANTS (VITAMINS C AND E AND OMEGA-3 OIL) AGAINST ALUMINUM CHLORIDE INDUCED BIOCHEMICAL CHANGES IN FEMALE ALBINO RATS (*RATTUS RATTUS NORVEGICUS*)

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Abstract

The present study was undertaken to evaluate the protective effect of certain antioxidants such as Vitamins C, E and Omega-3 oil on Aluminum induced biochemical changes in the female albino rats. Sixty four female adult rats were divided randomly into two control: (control 1) 0 $AlCl_3$ /Kg body weight (b.w.); (control 2) supplied orally with 0.2 ml/rat sun flower oil and six treated groups: $AlCl_3$ (60 mg/kg b. w.) ; $AlCl_3$ (60 mg/kg b. w.) plus 0.2ml/rat of 0.5% Acetic acid; $AlCl_3$ (60 mg/kg b. w.) plus Vit.C (50 mg/kg); $AlCl_3$ (60 mg/kg b. w.) plus Vit.E (100 mg/kg); $AlCl_3$ (60 mg/kg b. w.) plus 0.2 ml/rat of 5% Omega-3 and $AlCl_3$ (60 mg/kg b. w.) plus Vit.C (50 mg/kg) plus Vit.E (100 mg/kg) plus 0.2ml/rat of 5% Omega-3) respectively. Rats were orally administered their respective doses every other day for 35 days. At the end of the experiments, body weights were recorded and blood samples were collected for biochemical tests. Rats treated with aluminum chloride in the presence or absence of acetic acid showed significant decreases in the rate of body weight gain as compared with the control. Antioxidants (Vitamins C, E and omega-3) along with aluminum chloride produced protective effects as the rate of body weight gain approximately was more or less similar to the normal values of the control.

The rats treated with $AlCl_3$ (in the presence or absence of acetic acid) showed a significant increase in Alanine aminotransaminase (ALT), Aspartate aminotransaminase (AST), Urea and Creatinine and a significant decrease in serum albumin and total protein as compared with the control. The administration of antioxidants (Vit C, E and Omega-3 oil) along with $AlCl_3$ showed protective effects on liver and kidney since ALT, AST, Urea, Creatinine, albumin and total protein were tending to return towards their normal levels of control. So, the present study showed that Vit C, E and Omega-3 oil can be effective in the protection of aluminum-induced toxicity.

***Key Words**: Al toxicity, Vitamin C, Vitamin E, Biochemical, Enzymes.*

Introduction

Aluminum (Al) is the third most abundant element comprising approximately 8% of the earth's crust (Klein, 1991). The main sources of Al include corn, yellow cheese, salt, herbs, spices, tea, cosmetics, and Al cooking utensils (El-Demerdash *et al*., 2004 and Yousef, 2004). In addition, Al compounds are widely used in medicines such as antacids, phosphate binders, buffered aspirin, vaccines and allergen injections and fluids used in renal dialysis (Kaehny *et al*., 1997 and Yokel, 2004).Normal adults consume approximately 3-5 mg Al in daily with the diet and variable amounts from drinking water depending on local conditions including alum treatment and acidification (Nordberg *et al*., 1985). Aluminum sulphate is the most widely used coagulant for clarifying turbid drinking water (Martin, 1986; Ochmanski and Barabasz, 2000).

Aluminum is absorbed through the skin, gastrointestinal tract, lung, and nasal mucosa. After absorption, most Al is transported by the blood to various body organs. Bone, muscle and lung contain the highest Al contents in the normal human being. Al uptake by the brain is linked to the presence of high affinity of transferring receptors (Anane *et al*., 1997).Aluminum is also accumulates in a number of mammalian tissues, including kidney, liver, brain and bone (Anand *et al*., 2002). Al accumulation in the kidney promotes the degeneration of the renal tubular cells, and inducing nephrotoxicity (Mansour *et al*., 2006). Therefore, Al accumulation in the kidney promotes renal failure and the subsequent systemic toxicity (Mahieu *et al*., 2005). Also, Al accumulation in the liver leads to cholestasis (Osinska *et al*., 2004).The toxicological effects of Al on humans include encephalopathy (Alfrey *et al*., 1976), bone disease (Ward *et al*., 1978), anemia (Short *et al*., 1980) and skeletal system disease (Gupta *et al*., 2005).It may also be a contributing factor for the development of Alzheimer's disease (AD) (Campbell, 2002). These toxic effects of Al have been suggested to be due to the generation of reactive oxygen species (El-Demerdash, 2007), which results in

the oxidative deterioration of cellular lipids, proteins, and deoxyribonucleic acid (DNA) (El-Demerdash 2004; Mansour *et al*., 2006). So, these toxic effects of Al appear to be mediated, at least in part, by free-radical generation (Moumen *et al*., 2001; Anane and Creppy, 2001).

Cronan and Schofield (1979) have shown at neutral pH, Al minerals are insoluble, but solubility increases at lower pH. Thus, acidification of lakes and streams by acid rain mobilized Al from the soil to the aquatic environment. The levels of dissolved Al in water are strongly influenced by pH and the presence of other substances in the water (Browne *et al*., 1990).Some studies were carried out to evaluate the potential protective role of antioxidant vitamins, such as vitamin C, vitamin E (Yousef *et al*., 1999; Salem *et al*., 2001).

Vitamin C (Vit.C) (ascorbic acid) is an essential micronutrient required for normal metabolic functioning of the body. Many biochemicals, clinical and epidemiological studies showed that vitamin C may be of benefit in chronic diseases such as cardiovascular disease, cancer and cataract, probably through antioxidant mechanisms (Carr and Frei, 1999).

Vitamin E (Vit.E) (α-tocopherol) is a naturally occurring antioxidant nutrient that has an important role in animal health through the inactivation of harmful free radicals that are produced during normal cellular activity and under various stress conditions (El-Demerdash ,2007 ; Yousef, 2004). The antioxidant functions of this micronutrient, also, at least in part, enhance immune reactions by maintenance of the functional and structural integrity of the all-important immune cells (Yousef *et al*., 2003; El-Demerdash *et al*., 2004).

Omega-3 poly unsaturated fatty acid from fish and fish oil can protect against chronic heart disease (CHD), both health professional and publics are increasingly interested in its role in the prevention and management of CHD. During multiple pharmacological treatments for cardiovascular disease, many researchers believed that dietary intervention or nutritional supplements may be a more natural and acceptable method of providing benefits (Garrido-Sanchez *et al.,* 2008). The current work aimed to study the effect of $AlCl_3$ on some biochemical parameters and the protective effects of some antioxidants (Vitamins C and E and Omega-3 oil) on Al induced biochemical changes of liver and kidney tissues.

Materials and Methods

Experimental animals

Adult female albino rats *Rattus rattus norvegicus* were used during the present study. The rats were 10-12 weeks old with a body weight ranging from 190-210 g. The rats were kept in polypropylene rat's cages at a rate of 2 animals per cage. The cages were bedded with wood chips and the animals had free access to standard rodent diet and tap water *ad libitum*. The animals were kept in animal house of biology department (Faculty of Science, University of Zakho), maintained under laboratory conditions at a controlled temperature of about 24± 2 °C and exposed to a photoperiod of 12 hrs light followed by 12 hrs of darkness. Animals were acclimated to the laboratory condition for about 7 days before the application of experimental work.

Experimental design

Sixty four adult female albino rats were used in this study. The rats were divided randomly into eight groups, each of eight individuals and treated as in (Table 1).

Table (1): The distribution of rats in their experimental groups. b.w., body weight; G, treatment groups.

Groups	Number of Rats	Dose	Duration
G1: Control	8	----------	35 days
G2: Control 2	8	0.2 ml Oil/rat	35 days
G3: Aluminum chloride	8	60 mg/kg b.w.	35 days
G4: $AlCl_3$ + Acetic acid	8	60 mg/kg b.w. + 0.2 ml 0.5% Acetic acid	35 days
G5: $AlCl_3$ + Vitamin C	8	60 mg/kg b.w. + 50 mgVit.C/kg b.w.	35 days
G6: $AlCl_3$ + Vitamin E	8	60 mg/kg b.w. + 100 mgVit.E/kg b.w.	35 days
G7: $AlCl_3$ + Omega-3	8	60 mg/kg b.w. +5% Omega-3	35 days
G8: $AlCl_3$ + Vitamin C + Vitamin E + Omega-3	8	60 mg/kg b.w .+ 50 mgVit.C/kg b.w. + 100 mgVit.E/kg b.w .+ 5% Omega-3	35 days

The doses of $AlCl_3$, Vit. C and Vit. E were calculated according to the animal's body weight before their uptake .The desired doses of $AlCl_3$, Vit. C, Vit. E, Acetic acid and Omega-3 for each animal were daily intubated into oesopharyngael region daily, using small syringe connected to thin silicon tube.

Total body weight

Total body weight for each animal was measured and recorded twice; first at the beginning of the experiment and second at the end of the experiment using a top loading balance (Adventure ^{Tm}OHAMUS, USA). Finally, the rate of body weight gain was calculated.

Serum biochemical analysis

The blood sample was taken from the rat by heart puncture and withdrawn into a dry and clean non- heparinized tube. The sample was allowed to clot at room temperature for 30 minutes. Then the sample was centrifuged at 3000 rpm for 15 minute (Dacie and Lewis, 1984). Serum samples were placed in eppendrof tubes and used for determination of some biochemical parameters such as serum ALT, AST, Albumin, Total protein, Urea and Creatinine by using Auto Analyzer Spectrophotometer (Model Lisa Xs-French).

Statistical analysis

For body weight and serum biochemical parameters, all data were expressed as mean ± standard error (M ± S.E.) and statistical analysis was carried out using statistical available software (SPSS version 17.0). One way analysis of variance (ANOVA) was performed to test for significance followed by Duncan's multiple range comparison tests for comparison between the groups. P values (0.05) and (0.01) were considered significant.

Results

Effects of $AlCl_3$ alone or along with acetic acid and some antioxidants on the body weight gain.

As shown in Table (2) rats treated with $AlCl_3$ and $AlCl_3$ plus acetic acid have shown a significant decrease ($P<0.05$) in the rate of body weight gain as compared with the control. On the other hand, rats treated with $AlCl_3$ plusVit.C, $AlCl_3$ plus Vit.E, $AlCl_3$ plus omega-3 and their combinations in comparison with control and control 2 did not produced any significant ($P > 0.05$) reduction in the rate of body weight gain.

Table (2): Effects of $AlCl_3$ along with acetic acid, and some antioxidants on the body weight gain of rats.

Groups	Monthly Body Weight Gain(gm) % Mean ± S.E.
Control	11.204±1.109[b]
Control 2	10.276±1.308[b]
$AlCl_3$	2.651±1.201[a]
$AlCl_3$ + Acetic acid	2.462±1.016[a]
$AlCl_3$+Vit.C	10.347±1.022[b]
$AlCl_3$+Vit.E	8.988±1.772[b]
$AlCl_3$+Omega-3	8.48±1.76[b]
$AlCl_3$+Vit.C+Vit.E+Omega-3	8.241±1.557[b]

Note: Different letters represent the presence of a significant difference ($P<0.05$).

Effects of $AlCl_3$ and some antioxidants on serum ALT.

As illustrated in Table (3) rats treated with $AlCl_3$ and $AlCl_3$ plus acetic acid have shown a significant increase ($P<0.01$) in serum ALT as compared with control. On the other hand, serum ALT activity in rats treated with $AlCl_3$ plus Vit.C, $AlCl_3$ plus Vit.E, $AlCl_3$ plus Omega-3 and their combinations was not influenced by Al and they showed approximately normal ALT activity which was statistically non-significant when compared with that of the control ($P>0.05$).

Effects of $AlCl_3$ and some antioxidants on serum AST.

Rats treated with $AlCl_3$ in the presence of acetic acid showed a significant increase ($P<0.01$) in AST activity as compared with the control (Table 3). The presence of individual antioxidants along with Al showed a mild protective effect of body organs, since AST activity was still elevated but to a lesser extent as compared with Al treated rats. However, a combination of Vit.C and E and Omega-3 along with Al showed much better protective effect as indicated by more or less normal AST activity.

Effects of $AlCl_3$ and some antioxidants on serum Total Protein.

As the results indicate, aluminum in the presence or absence of acid significantly reduced the level of total protein ($P < 0.05$) when compared with control. On the other hand, in rats supplied with Vit.C and E and Omega-3 and their combination showed protective effects on serum total protein since its level was closely similar to its normal level as shown in table (3).

Effects of $AlCl_3$ and some antioxidants on serum Albumin.

In rats treated with $AlCl_3$ and with $AlCl_3$ plus acetic acid had shown a significant reduction ($P<0.01$) in the level of albumin as compared with those of the control rats. On the other hand, the level of Albumin in rats treated with Vit. E and Omega- 3 and their combinations along with Al returned to more or less to normal values and showed non-significant differences as compared with the control ($P> 0.01$). However, in rats treated with Vit. C along with Al, the level of albumin was significantly reduced ($P < 0.01$). As shown in table (3).

Effects of $AlCl_3$ and some antioxidants on serum Urea.

As the results indicated, rats treated with Al in the presence or absence of acid produced a mild and statistically non significant elevation ($P>0.05$) in the level of serum urea. Furthermore, administration of Vit.C and E and Omega-3 returned the level of serum urea toward the control level as shown in table (3).

Effects of $AlCl_3$ and some antioxidants on serum Creatinine.

The results of the experiments on the effect of $AlCl_3$ alone or with acid, Vit.C and E and Omega-3 on serum creatinine level are showed in Table (3). As the results indicate, $AlCl_3$ alone caused a highly significance ($P<0.01$) elevation in the level of creatinine as compared with the control. On the other hand, administration of Vitamin C, E, and Omega-3 and their combinations showed a protective effect on the kidney as indicated by the exhibition of approximately the control creatinine values.

Table (3): Effect of $AlCl_3$ alone or along with acetic acid and some antioxidants on some biochemical parameter.

Groups	SALT ** (IU/L)	SAST ** (IU/L)	T. Protein * (g/dl)	Albumin ** (g/dl)	Urea * (mg/dl)	Creatinine ** (mg/dl)
1.Control	64.666±2.027 ^{a}	129.333±1.855 ^{a}	6.333±0.088 ^{b}	2.533±0.176 ^{b}	48.666±1.333 $_{a}$	0.556±0.0088 ^{a}
2.Control 2	82.800±12.866 ^{a}	149.800±13.990 ^{ab}	6.620±0.086 ^{b}	2.568±0.021 ^{b}	51.400±2.204 $_{a}$	0.554±0.0067 ^{a}
3.$AlCl_3$	118.750±8.097 ^{b}	167.200±26.946 ^{ab}	5.797±0.178 ^{a}	2.230±0.068 ^{a}	54.400±2.400 $_{a}$	0.688±0.0546 $_{b}$
4.$AlCl_3$+ Acetic acid	122.600±13.786 ^{b}	195.000±8.955 ^{b}	5.820±0.124 ^{a}	2.152±0.024 ^{a}	50.400±2.336 $_{a}$	0.626±0.0222 $^{a\ b}$
5.$AlCl_3$+ Vit.C	97.600±2.227 ^{ab}	157.200±10.165^{ab}	6.400±0.192 ^{b}	2.868±0.069 ^{c}	49.000±0.707 $_{a}$	0.550±0.0204 ^{a}
6.$AlCl_3$+ Vit.E	93.166±3.544 ^{ab}	159.000±9.295 ^{ab}	6.475±0.170 ^{b}	2.653±0.057 ^{bc}	48.000±0.577 $_{a}$	0.540±0.0200 ^{a}
7.$AlCl_3$+ Omega-3	92.400±5.045 ^{ab}	161.666±3.343 ^{ab}	6.520±0.106 ^{b}	2.746±0.036^{bc}	47.400±2.785 $_{a}$	0.528±0.0073 ^{a}
8.$AlCl_3$+ Vit.C+Vit.E+Omega-3	90.000±2.594 ^{ab}	120.666±4.835 ^{a}	6.516±0.203 ^{b}	2.804±0.046 ^{bc}	48.333±2.260 $_{a}$	0.520±0.0089 ^{a}

The values represented by mean ± S.E. of Mean, N=8, Duncan's test used to compare between groups,similar letters in the same column refers to non significant level while different letters represent to significant level: ** ($P<0.01$) and * ($P<0.05$).

Discussion

In the presented study, oral administration of $AlCl_3$ in the presence or absence of acetic acid for 35 days significantly reduced the rate of body weight gain as compared with the control groups. These results agree with those observed by Sallam *et al.*, (2005) in rats treated with 34mg /kg $AlCl_3$. This reduction in the rate of body weight gain may be due to the elevation of malonaldehyde level by heavy metals and a reduction in the levels of both glutathione and catalase. Variation in the activity of these enzymes may contribute in the maintenance of lipid peroxidation induced by the metals (Corpas *et al*., 2002). Furthermore, partial disruption of small intestine villi and subsequent malabsorption of nutrients represents another factor that may be responsible for the loss of body weight (Al-Qudah, 2006). This reduction in nutrients transport causes an inhibition in adenosine tri phosphate (ATP) production, active transport in amino acid and subsequent inhibition in protein syntheses (John 1982).

In this study, rats treated with some antioxidants (vitamins C and E and omega-3) along with aluminum, the rate of body weight gain increased as compared with that animals treated with Al in the presence or absence of acid. This may be due to the antagonists effect of above vitamins on the toxic effect of Al and subsequent protection of the body from Al-toxicity (Yousef, 2004 and El-Demerdash, 2007). Furthermore, the antioxidants effect of omega-3 also reduces the aluminum toxicity (Mete *et al*., 1999).

In the current study, treatment of rats with $AlCl_3$ or $AlCl_3$ with acetic acid significantly increased the activities of both serum ALT and AST. The toxic effect of Al was enhanced in the presence of acid. These results agree with those reported by Al-Sulaivany (2010) in rats treated with Al in the presence or absence of acid. He found significant elevation in serum ALT and AST activities and reduction in their activities in liver and kidney tissues. This indicates that increase in serum enzyme activity is resulted from the leak of the enzyme from body tissues and organ including liver and kidney tissues. Similar results were also observed by Hassoun and Stoths (1995), Chinoy and Memon (2001) and El-Demerdash (2007). They indicated that exposure to Al caused liver necroses and subsequent escape of AST from them to the blood. Furthermore, the increase in ALT level is resulted from the cellular destruction of the body tissues including the liver (Harper *et al*., 1979).

The presences of vitamins C, E, omega-3 and their combinations along with Al alleviated the toxicity of Al on body tissues since the activities of both ALT and AST tended to return back approximately to the normal levels. Furthermore, combinations of omega-3, vitamins C and E greatly reduced the toxic effect of Al as indicated by the return of the activities of these enzymes to their normal values. Due to the availability of limited information about the protective effects of antioxidant on Al toxicity in rodents, it is difficult to compare the results. However, Al-sulaivany (2010) observed more or less a similar protection effect of antioxidants on ALT and AST in the tissues of rats exposed to Al. Also a similar reduction in the toxic effect of heavy metals in the presence of antioxidants was observed by Tawwab *et al*. (2004).

In this study, rats treated with Al in the presence or absence of acid significantly elevated the level of serum creatinine. A similar elevation in urea and creatinine level in $AlCl_3$ treated rats was considered as a significant marker for renal dysfunction (El-Demerdash, 2007 and Al-Sulaivany, 2010). Szilagyi *et al*. (1994) reported that alteration in serum urea may be related to metabolic destruction (e.g. renal function, cation-balance… etc.) produced by heavy metals. In addition, Katyal *et al*. (1997) reported that Al has been implicated in the pathogenesis of several clinical disorders, including renal dysfunction. Increased urea concentrations in the plasma of animals treated with Al and Al plus acetic acid may be due to its effect on liver function, as urea is the end-product of protein catabolism, and/or referred to kidney dysfunction as indicated by enlargement of the relative weight of kidney. Decreased protein levels in Al-treated rats might be due to changes in protein synthesis and/or metabolism (Chinoy and Memon 2001).

Exposure of rats to $AlCl_3$ in the presence or absence of acid significantly reduced total serum protein and albumin. These results agree with those reported by Al-Sulaivny (2010) and Al-Demerdash (2004).Decreased serum protein in rats exposed to Al might be due to villi disruption and subsequent malabsorption and transport of nutrients (Al-Qudah, 2006). This was followed by depression of protein synthesis and metabolism (Chinoy and Memon, 2001).

The uptake of vitamins C, E and omega-3 produced a protective effective in Al treated rats

since the levels of total protein and albumin returned approximately to their normal values. Similar results were reported by Al-Sulaivany (2010) during administration of antioxidants to Al treated rats. Al intoxicated animals showed a number of indicators of oxidative stress, which includes increases in the level of Thiobarbituric acid reactive substances (TBARS) and decreases in Glutathione (GSH), Glutathione S-transferase (GST) and catalase in the rats testes (Yousef and Salama 2009). Al induced oxidation stress may be resulted from the generation of free radical (Gomez *et al*., 1997; Yousef, 2004; Yousef *et al*., 2005). However, Al is considered to be a non-redox active metal, it promotes biological oxidation both *in vitro* and *in vivo* because of its pro-oxidant activity (Gomez *et al*., 2005; Yousef *et al*., 2007; Turner and Lysiak, 2008). Increased reactive oxygen species (ROS) was reported in previous studies during Al exposure, which was attributed to electron leakage, enhanced mitochondrial activity and increased electron chain activity(Flora *et al*., 2003). Furthermore, they added that ROS subsequently attack almost all cell components including membrane lipids and producing lipid peroxidation. Therefore, it can be hypothesized that oxidative stress may be one of the contributing factors to Al-induced liver dysfunction (Yousef and Salama, 2009). Finally it was indicated that when rats treated with $AlCl_3$ had undergone a reduction in the body weight gain. These effects were counteracted on administration antioxidants and omega-3 along with $AlCl_3$. and serum biochemical parameters were returned to more or less normal values when rats were treated with antioxidants and omega-3 along $AlCl_3$.

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هه لسەنگاندنا کارتێکەرێت پاراستنا هندە ك كە رەستێت دژی ئە كسە دێ وەك فیتامین س و ی و زەیتامێلاکا نەهە نگی ل سەر گوهورینا چەند پارامیتەرێت خوینێ یێن جردێن سپی ئەوێت هاتینە توشکرن بو ئەلنیوم کلورایدی

کورتیا ڤە کولینێ

مەرەم ژ ڤێ ڤەکولینێ ئە وە هە لسەنگاندنا کارتێکەرێت پاراستنا هندە ك كە رەستێت دژی ئە كسە دێ وەك فیتامین سی و یی و زەیتامێلاکا نەهە نگی ل سەر گوهورینا چەند پارامیتەرێت خوینێ یێن جردێن سپی ئەوێت هاتینە توشکرن بو ئەلنیوم کلورایدی.

دڤێ ڤە کولینێدا ٦٤ جردێن سپی یێن گەهشتی کوژیێ وا دناڤبەرا ١٠-١٢ حەفتیاندا بوون وکێشاوا دناڤبەرا ١٩٠-٢١٠گرامادابون.ئەڤ جردە هاتنە خودان کرن ل ژورا گیانەوەرا ل پشکا بایولوجی,کولیژا زانست, لزانکویا زاخو لبن کاودانێن ستاندارد یین تاقیگەهی کوپلا گەرماتیێ ٢٤ پلەیە ودەمێ روناهیێ ١٢ دمژمێرن ,خارن وئاڤ دهاتنە بەرهەڤکرن روژانە تا دوماهیکا ڤەکولینێ.

ئەڤ جردێن هەنێ هاتنە دابەشکرن ل سەر ٨ کوما وەکی ل خوارێ دیارکری:-کوما ئیکێ(کونترول).

کومادووێ(کونترول 2 ,دانا ٠,٢ ملیێت زەیتا گولبەروژا بو هەر جردەکی بڕێکا دەڤی).کوماسیێ(دانا ٦٠ ملیگرامێت ئەلەمنیوم کلورایدی بوهەرکیلویەکی ژکێشا لەشی بڕێکا دەڤی).کوماچوارێ(دانا ٦٠ ملیگرامێت ئەلەمنیوم کلورایدی بوهەرکیلویەکی ژکێشا لەشی دگەل ٠,٢ملا ژ٠,٥٪ ژ ترشێ خەلیکی بڕێکا دەڤی). کوماپینجێ(دانا ٦٠ ملیگرامێت ئەلەمنیوم کلورایدی بوهەرکیلویەکێ ژکێشا لەشی دگەل٥٠ملیگرامێت ڤیتامین سی بوهەرکیلویەکی ژکێشا لەشی بڕێکا دەڤی). کوماشەشێ(دانا ٦٠ملیگرامیت ئەلەمنیوم کلورایدی بوهەرکیلویەکێ ژکێشا لەشی دگەل ١٠٠ملیگرامێت فیتامین ی بوهەرکیلویەکی ژکێشا لەشی بڕێکا دەڤی). کوماحەفتێ(دانا ٦٠ ملیگرامێت ئەلەمنیوم کلورایدی بوهەرکێلویەکی ژکێشا لەشی دگەل ٠,٢ملا بو هەر جردەکی ژ ٥٪ ژ زەیتامێلاکا نەهەنگی بڕێکا دەڤی). کوماهەشتێ(٦٠ملیگرامێت ئەلەمنیوم کلورایدی بوهەرکیلویەکی ژکێشا لەشێ دگەل٥٠ ملیگرامێت ڤیتامین سی بوهەرکیلویەکێ ژکێشا لەشێ دگەل ١٠٠ملیگرامێت فیتامین ی بوهەرکیلویەکی ژکێشا لەشێ دگەل ٠,٢ ملا بو هەر جردەکێ ژ ٥٪ ژ زەیتامێلاکا نەهەنگی بڕێکا دەڤی). لدوماهیا ڤەکولینی کوبوماوی ٣٥ روژا ڤەکێشا,کێشین جردا هاتنە تومارکرن,سامپلێت خوینێ هاتنە وەرگرتن.

ئەوجردێن هاتینە توشکرن بو ئەلنیوم کلوراید دگەل ترشێ خەلیکی یان ژی بتنێ دیاربوکو کێمبونەکا بەرچاڤ هەبو د کێشا لەشێن جردادا.بەلی دانا ئەوکەرەستێن دژی ئەکسەدێ وەك (فیتامین سی و ی و زەیتامێلاکا نەهە نگی) دگەل ئەلنیوم کلوراید دئەنجامدا ئەف کێشە زفراندن بو نێزیکی کونترولێ.

ئەوجردێن هاتینە توشکرن بو ئەلنیوم کلوراید دگەل ترشێ خەلیکی یان ژی بتنی دیاربو کوبلندبونەکا بەرچاڤ هەبو دئەنزیمێن AST,ALT وهەروسا بلندبونا یوریا وکریاتینینی دخوینێدا,دیسان کێمبونەکا بەرچاڤ هەبو د پروتینی وئەلبومینی خوێنیدا دەمێ ئەم بەراوردبکەین دگەل کونترولی.

دانا چەند کەرەستێن دژی ئەکسەدێ وەك(فیتامین سی و ی و زەیتامێلاکا نەهە نگی) دگەل ئەلنیوم کلوراید دیاربو کوئەنزیمێن AST,ALT دئاستێ سروشتیدابو وزڤرینا ئاستێن یوریا وکریاتینین و پروتین وئەلبومینی بوئاستێن سروشتی.

STUDY OF THE PARASITES OF THE LOCAL CHICKENS (*GALLUS GALLUS DOMISTICUS*) IN DUHOK PROVINCE, KURDISTAN REGION-IRAQ

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Abstract

One hundred and twenty chickens the local breed (*Gallus gallus domesticus*) were examined during the period from June to October 2012 in Duhok Province / Kurdistan Region of Iraq. To investigate the prevalence of ecto and endoparasites among them. The recorded parasites included: Two species of lice namely *Mencanths stramineus* with infestation rates of 34% and *Goniocotes gallinae,* with infestation rate of 0.8%. One species of soft tick, genus *Aragas persicus,* was also recorded, with an infestation rate of 4.1%. Whereas, the endoparasite examination revealed 16.6% from protozoa (*Eimeria*) and the presence of four nematodes and six cestode species, but no trematodes and blood parasites. The highest nematode infection rate was 46.6% with *Subulura* species, followed by *Ascaridia galli* with infection rate of 38.3%, *Heterakis gallinarum* with infection rate of 25% and, *Capillaria* with infection rate of 4.1%. Regarding cestodes, the recorded species included: *Raillietina tetragona, R. echinobothrida, R. cesticillus, Fimbriaria fasciolari, Davainea proglottina,* and *Amoebotaenia sphenoides* with infection rates of 27.5%, 24.1%, 6.6%, 1.6%, 2.5%, and 0.8%, respectively.

***Keywords**: Local chickens, ecto and endoparasites, lice, cestode, nematodes*

Introduction

The domestic fowls are the most important protein sources of human populations in every part of the world. It is demonstrated that during the last thirty years, eggs and poultry meat were constantly increasing (Kaingu *et al*., 2010). Parasitic infections of poultry are the major factors responsible for economic losses through reduction in productivity and increased mortality (Mirhadi *et al*., 2011). Poultry are subjected to a wide variety of diseases including Newcastle disease, salmonelosis, respiratory disease and a large number of ecto-endoparasites. Various ectoparasites are reported in the local fowls such as lice, fleas, mites and soft ticks (Urquhart *et al*., 1996). Gastrointestinal helminthes of poultry are commonly divided into three main groups: nematodes, cestode and trematodes. Nematodes are considered the most important group of helminthes of poultry (Bachaya *et al.*,2012) Limited work has been done on ectoparasites and endoparasites of fowls in Iraq including Kurdistan Region, therefore, this study aimed to investigate the ecto and endoparasites of the Domestic fowl *Gallus gallus domisticus* in Duhok Province.

Materials and Methods

This study was carried out in Duhok province – Kurdistan Region of Iraq, during the period from June to October 2012, to investigate the presence of ecto and endoparasites of the local chickens were randomly selected and purchased from different parts of the Duhok city and its surrounding areas such as Shinkal, Semel, Aqra, Faida and other area in the Animal's House Laboratory of the Faculty of Medical Sciences / Duhok University.

One hundred and twenty live indigenous breed chickens (*Gallus gallus domesticus*) of both sexes (50 hens and 70 cocks) and different ages of free range breeding were purchased from local markets randomly, and examined for ecto and endoparasites.

Examination of Chickens

1-) Antemortem examination and collection of ectoparasites

The whole body of each chicken, including the skin and the feathers, was examined by the naked eye and with the aid of magnifying lens for the presence of ectoparasites (Moyo, 2009). The ectoparasites were collected gently using thumb forceps throw inflammatory lesion of skin scraped, these samples were mixed with 10% KOH, and from the feather by spraying with commercial insecticide after that ectoparasites were preserved in a test tube containing 70% ethyl alcohol until the time of identification. The

ectoparasites were examined under dissecting microscope and identified according to keys described by Soulsby (1982).

2-) Postmortem Examination

Following slaughtering of each chicken, the blood samples were collected directly in a sterile test tube containing EDTA anticoagulant for thin blood film were stained with leishman's stain for the presence of blood parasites.

The trachea, abdominal and thoracic cavity were opened followed by incised the esophagus, crop, gizzard and proventiculus, then small intestine (duodenum and ileum). Each part was incised longitudinally; visible worms to the naked eye were picked up using thumb forceps and the contents were examined by gross examination for the presence of worms The observed helminthes were washed with physiological saline and then fixed with warm 70% alcohol.

Nematodes were cleared in lactophenol and examined for morphology under the light microscope at 10X magnification. Identification of helminthes was based on the helminthological keys (Soulsby, 1982), while cestode were stained with Carmine stain.

Intestinal contents were also examined by flotation methods for the presence of coccidian oocysts. Only the genus of coccidian oocysts was identified according to morphological features (Soulsby, 1982), and scraping from the intestinal mucous were taken and examined for *Cryptosporidium* Oocysts identification.

3-) Statistical Analysis

Chi-square ($\chi 2$) test was used to analyze the association between prevalence and the explanatory variables such as age, sex, and management system. In all the cases, $p < 0.05$ were considered to be statistically significant (Tesfaheywet *et al.*, 2012).

Results and Discussion

Table(1) shows the percentage distribution of parasitic species identified in 120 local breed chickens examined in this study 83.3% of the chickens were found to be infected with external and internal parasites. The percentage of infection with internal parasites was 44.1%, while 9.1% was with external parasites and 30% with mixed infection.

Table (1): The percentage of infection with ecto and endoparasites and mixed parasites among examined chickens (No. 120).

Type of parasites	No. of infected chickens	%
External parasites	11	9.1
Internal parasites	53	44.1
External and internal parasites	36	30
Total No. infected	100	83.3

Regarding the sex, 84.2% of the female chickens were infected, while 82% of the male chickens were infected, but statistically the difference in the rate of infection in both sexes was non-significant (P>0.05) as summarized in Table (2).

Table (2): The percentage of infection with ecto and endoparasites among examined chickens, according to sex.

Sex	No. of examined chickens	No. of infected chickens	%
Female chickens	70	59	84.2
Male chickens	50	41	82
Total	120	100	83.3

Non- significant (P> 0.05) P value = 0.9204

Degree of Freedom: 1 $\chi 2$: 0.009995

The highest percentage (88.4%) of infection was among the chick group, while the percentages of infection in adult and growing groups were slightly lower (86.2%, and 75%, respectively), but statistically there were no significant differences (P>0.05) between parasitic infections and age groups as shown in Table (3)

Table (3): The percentage of infection with ecto and endoparasites among the examined chickens according to age groups.

Age groups	Age / months	No. of examined chickens	No. of infected chickens	% of infection
Adult	> 8	58	50	86.2%
Growing	2 - 8	36	27	75%
Chick	< 2	26	23	88.4%
Total		120	100	83.3

Non-significant (P> 0.05) P value= 0.6203 Degree of Freedom: 4 $\chi 2$: 2.637

Ectoparasites:-

The percentage of infestation with ectoparasites among all of the examined chickens was 39.1%.The results showed that 2 species of lice were identified. These species were *Mencanths stramineus* and *Goniocotes gallinae* with infestation rates of 34% and 0.8%, respectively. Regarding the soft ticks, one species was recorded, which was *Aragas persicus* with infestation rate of 4.1%

as summarized in Table (4).

Table (4): The percentage of infestation with ectoparasites among all infested chickens.

Type of parasites	Species of parasites	No. of positive	% infested with External parasites (47)	% of examined chickens (120)
Lice	*Mencanths stramineus*	41	87.2	34
	Goniocotes gallinae	1	2.1	0.8
Soft ticks	*Aragas persicus*	5	10.6	4.1

Endoparasites:-

The current study revealed that none of the blood parasites were observed in all of the examined chickens as shown in Table (5). Regarding the endoparasites different types of *Eimeria* oocysts with infection rate of 16.6% were recorded in this study (table.5).

Table (5): The percentage of infection with *Eimeria* oocysts among total examined chickens.

parasites	No. of examined chickens	No. of positive	%
Protozoa (*Eimeria* oocysts)	120	20	16.6

The percentage of infection with helminthes among the total number of examined chickens is listed in Table (6) according to their classes.

Table (6): The percentage of infection with different types of helminthes according to their classes among the examined chickens (No.120).

Type of parasites	No. of positive	% of infection
Nematodes	44	36,6
Cestodes	11	9.1
Nematodes + Cestodes	34	28.3
Trematodes	0	0

According to the percentage distribution of nematodes, the highest percentage of infection was 46.6% with *Sublura*, followed by *Ascaridia galli* which was 38.3%, *Heterakis gallinarum* with infection rate of 25% and *Capillaria* sp. with infection rate of 4.1% as summarized in Table (7).

Table (7): The percentage of infection with intestinal nematodes among the Examined chickens (No.120).

Species of parasites	No. of positive	% of infection
Ascaridia galli	46	38.3
Heterakis gallinarum	30	25
Sublura Spp	56	46.6
Capillaria Spp	5	4.1

Six species of tapeworm were recorded and identified, which were: *Raillietina tetragona*, *R. echinobothrida*, *R. cesticillus*, *Fimbriaria fasciolaris, Davainea proglottina*, and *Amoebotaenia sphenoides* with percentage distribution of 27.5%, 24.1%, 6.6, 1.6%, 2.5%, and 0.8%, respectively Table (8).

Table (8): The percentage of infection with intestinal cestode among the examined chickens (No.120).

Species of Cestode	No. of positive	% of infection
Raillietina tetragona	33	27.5
Raillietina echinobothrida	29	24.1
Raillietina cesticillus	8	6.6
Fimbriaria fasciolaris	2	1.6
Davainea proglottina	3	2.5
Amoebotaenia sphenoides	1	0.8

The results of the present study demonstrated a high rate of infection (83.3%) in the local breed chickens while Eslami *et al*. (2009) in Iran reported a higher rate (96%) of infection in the free-range chickens. Regarding the sex of the infected chicken female chickens had a slightly higher infection in comparison to male chickens this result is in agreement with the findings of Matur *et al.* (2010) in Nigeria during their studies of 500 gastrointestinal tracts of native and exotic breeds of chickens. The relation between parasitic infection and age groups of the present study,is in line with that of Tesfaheywet *et al.* (2012) in Southeastern Ethiopia in which they recorded slight difference in infection rates of examined chickens among chicks, growers and adults which were 38.0%, 37.6% and 45.9% ,respectively. High infection rate was recorded with ectoparasites similarly AL- Hubaity (1976) in Mosul; Ashenafi and Yimer, (2005) in central Ethiopia also recorded high rates of infection with ectoparasites. A high rate of infection (89%) with endoparasites was recorded in this study, this rate is much higher than the rates

recorded by other researchers as they recorded rates ranged from 4.3% to 36% of infection with endoparasites of domestic fowl (Sayyed *et al.*, 2000; Muhairwa *et al.*, 2007). Regarding the blood parasites, the present study did not show any species of blood parasites in all of the examined chickens. This result disagrees with those found by Sabuni *et al*. (2010) in Kenya who found high infection (79.2%). In the current study, only 20 (16.6%) were positive for coccidian oocysts. While higher rate of infection with coccidian have been reported by Bachaya *et al*. (2012) in Pakistan, they recorded a rate of 59.6%. With respect to cestodes,similar results were reported by Eslami *et al.* (2009) in Iran, they found that *A. galli*, *H. gallinrum* and *Raiellietina species* were the most prevalent helminthes species. The same results were reported in chickens of Ethiopia and India (Yadav and Tandon, 1991) Trematodes were not found in this study, the reason may be due to the absence or limited spreading of the snail the intermediate hosts responsible for the transmission of trematodes in the studied areas.

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پوختە

ئەڤ ڤەکولینە هاتیە ئەنجامدان ژ پێخەمەت دیارکرنا مشەخورێن دەرەکی و ناڤخویی یێن تووشی مریشکا ژ جورێ *Gallus gallus domesticus* دبن، کو ب شێوەکێ خومالی دهێنە خودانکرن ل ناڤ باژێرێ دهوکێ \ هەرێما کوردستانا عیراقێ. هاتیە ئەنجامدان د ماوێ دناڤبەرا خزیرانا ۲۰۱۲ هەتا چریا دوێ یا هەمان سالْ.

بۆ ڤێ مەرەمێ، ۱۲۰ مریشکێن خومالی ژ هەردوو رەگەزان هەردیسان هەر ژ ئەنجامێن هاتینە تومارکرن دڤێ ڤەکولینێ دا ب رێژا ۳٤ ٪ و ۰,۸٪ ل دویڤ *Mencanths stramineus* و *Goniocotes gallinae* ئێك. هەردیسان جورەکێ پلپێلکا نەرم *Aragas persicus* ب رێژا ٤,۱٪.

هەردیسان تێستێن مایکروسکوپی بۆ پیساتیا هندەك ماددەییّن ژ ناڤپوشێ ریڤیکێن وان هاتینە وەرگتن هاتە کرن بۆ دیارکرنا جورێن جودا جودا ییّن هێکێن ئیمیری Eimeria ooc و رێژا تووشبونێ ۱٦,٦٪ دڤێ ڤەکولینێ دا چ حالەتێن تووشوبنێ ب مشەخورێن خوینێ blood parasites نەهاتنە تومارکرن ژ سەرجەمێ وان هەمی مریکشکێن هاتینە تاقیکرن.

چوار پولێن جودا جودا ییّن کرمێن نیماتودا ییّن ریڤیکان هاتنە توماکرن ژ سەرجەمێ مریشکێن تووشیوی، هەردیسان بلندرتین رێژا کرمێن نیماتودا ژ جورێ *Sublura* ب رێژا ٤٦,٦ ٪ و پاشی کرمێ *Ascaridia galli* ب رێژا ۳۸,۳ ٪ و پاشی کرمێ *Heterakis gallinarum* ب رێژا ۲۵ ٪ و دوماهیێ ژی کرمێ *Capillaria* ب رێژا ٤,۱ ٪. دەربارەی رێژا کرمێن شریتی ییّن تووشی ریڤیکێن مریشکێن خومالی بووین، دڤێ ڤەکولینێ دا شەش جورێن ڤان کرمان هاتنە تومارکرن ئەوژی ئەڤەبوون *Raillietina tetragona*، *R. echinobothrida*، *R. cesticillus* , *Fimbriaria fasciolaris*، *Davainea proglottina* و *Amoebotaenia sphenoides* ب رێژێن ۲۷,۵٪ , ۲٤,۱٪ , ٦,٦٪ ,۱,٦٪ ,۲,۵٪ و ۰,۸٪ ل دویڤ ئێك. دماوێ ئەنجامدانا تاقیکرنان لسەر ئەندامێن نافخویی ییّن مریشکان چ جورێن کرمێن پەحن Flat Worm نەهاتنە دیتن.

الخلاصة

أجريت هذه الدراسة لغرض تحديد الطفيليات الخارجية والداخلية التي تصيب الدجاج الأليف من نوع *Gallus gallus domesticus* المربى محليا في مدينة دهوك /إقليم كردستان العراق. وكانت فترة العمل من حزيران إلى تشرين الثاني ۲۰۱۲. تم فحص ۱۲۰ دجاجة محلية من كلا الجنسين سجل في هذا البحث، نوعين من القمل هما *Mencanths stramineus* و *Goniocotes gallinae* بنسبة إصابة ۳٤% و ۰،۸% على التوالي، ونوع واحد من القراد اللين (Soft ticks) بنسبة إصابة ٤،۱%. وكانت نسبة الاصابة بالأوالي (*Eimeria*) ۱٦،٦% من مجموع الدجاج المفحوص.

لم تسجل في الدراسة الحالية أي إصابة بطفيليات الدم blood parasites وأكياس الـ *Cryptosporidium* في جميع الدجاج المفحوص.

تم تسجيل أربعة أنواع مختلفة من الديدان الخيطية المعوية و أعلى نسبة للديدان الخيطية كانت بدودة *Sublura* بنسبة إصابة٤٦،٦% تلتها دودة *Ascaridia galli* ۳۸،۳% ثم دودة *Heterakis gallinarum* ۲۵% وأخيرا دودة *Capillaria* بنسبة إصابة ۰،۸%. أما بالنسبة للديدان الشريطية التي وجدت في أمعاء الدجاج المحلي في هذه الدراسة حيث تم تسجيل ستة أنواع من هذه الديدان وهي كالأتي *Raillietina tetragona*، *R. echinobothrida*، *R. cesticillus*، *Fimbriaria fasciolaris*، *Davainea proglottina* و *Amoebotaenia sphenoides* وبنسب إصابة ۲۷،۵%،۲٤،۱%،٦،٦%،۱،٦%،۲،۵% و۰،۸% على التوالي. ولم يتم عزل أي نوع من أنواع الديدان المسطحة (Termatode) في جميع الدجاج المفحوص.

DETERMINATION OF IONS AND IRON CONTENT IN HYDATID CYSTS OF *ECHINOCOCCUS GRANULOSUS* ISOLATED FROM DIFFERENT INTERMEDIATE HOSTS (SHEEP, GOATS, CATTLE AND HUMAN) TISSUES

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Abstract

This study included comparative biochemical composition of hydatid fluid, protoscolices, infected and non-infected tissues isolated from liver and lungs of infected sheep, goats, and cattle in Duhok abattoirs during the period from Nov. 2009 to Apr. 2010. Also hydatid fluid of cysts surgically removed from humans in Azadi Teaching Hospital, Duhok during the period from Mar. 2010 to Jul. 2010.Hydatid cysts and host tissues were analyzed for Ions (Na^+, K^+, Ca^{++}, and Mg^{++}) and Fe^{++}. Among Ions, Na+ exhibited high levels in hydatid fluid of the studied hosts with the highest being in hydatid fluid of sheep liver cyst (356±8.207 mg/dl); furthermore, infected tissues showed higher Na^+ levels with the highest being in sheep liver and lung tissues (196±7.461 and 178±5.868 mg/100g respectively). Protoscolices of both liver and lungs showed high K^+ levels, among tissues, infected tissue contained high K^+ levels with the highest being in infected lung tissues (Ranged from 63.46±0.597 mg/100g to 77.39±0.729 mg/100g). Nearly similar levels of Ca^{++} were detected in hydatid fluid and protoscolices of all cysts with the highest level being in goats cysts protoscolices (Liver: 9.212±0.081 mg/100g, Lungs: 9.044±0.072 mg/100g) and the lowest in cattle cysts fluid (Liver: 6.954±0.061 mg/dl, Lungs: 6.826±0.054 mg/dl). The level of Mg^{++} was low in hydatid cysts and tissues of all hosts (Ranged from 0.746±0.016 mg/100g to 0.952±0.015). Small quantities of iron were detected in hydatid cysts of all hosts which were higher in cattle liver cysts (Hydatid fluid 0.0882±0.003 mg/dl and protoscolices 0.0838±0.003 mg/100g) and infected liver tissues (0.2293±0.008 mg/100g).

KEYWORDS: *Echinococcosis, Echinococcus granulosus, HCs chemical composition, Ions, Iron.*

NTRODUCTION

Hydatid disease, Hydatidosis or Cystic Echinococcosis (CE) is a major parasitic disease of veterinary and public health importance throughout the world. This disease has a great economic and zoonotic importance because it affects almost all the domestic animals and human (Baswaid, 2007 and Surhio *et al*., 2011).

Cystic Echinococcosis is caused by the larval stage (Metacestod) of the dog tapeworm *Echinococcus granulosus* by ingesting eggs, which passed with the feces of the definitive hosts, usually feral dogs (Rahimi *et al*., 2011). Humans become infected accidentally through ingestion of eggs, mainly children who are often found infected, because of their closer contacts with dogs (Eckert and Deplazes, 2004).

The distribution of CE is normally associated with underdeveloped countries, especially in rural communities, where man maintains close contact with the dog, the definitive host and various domestic animals which may act as intermediate hosts (Eslami and Hosseini 1998). The larval form of *E. granulosus* in the intermediate host is characterized by its cystic aspect and behaves as a benign tumor in most of the cases (Amman and Eckert, 1996). The cysts may be single or multiple; their main locations are the liver and the lungs; however any other tissue or organ may be involved, including brain, bone, spleen, kidney, …etc. (Moro and Schantz, 2009).

There are seven species of *Echinococcus*, four of them are infectious to human, namely *E. granulosus, E. multilocularis, E. oligartharus* and *E. vogeli* (WHO,2001).

However *E. granulosus*, is the most prevalent species in all continents, causing considerable public health problems in many regions of the world (WHO, 2001). Furthermore, it is also common in Iraq (Al-Fatalawei, 2002 and Al-Nakeeb, 2004), including Kurdistan region (Ghaffar, 2008; Abdullah, 2010 and Meerkhan, 2011).

Epidemiological situation for this parasite is complicated by the fact that several strains have been identified in most area where infection is endemic. These strains exhibit different degrees of infectivity for certain intermediate hosts. Previously, strains were identified using morphological, biological, biochemical and

some other criteria. However, in recent years molecular techniques have contributed in more precise strain identification at the DNA level (Thompson and McManus, 2001).

Most of the studies on this parasitic disease focus either on the veterinary and zoological aspects of the parasite life cycles or on the prevention and control measures or on the medical aspects of the diseases and the treatment options in humans. The information about the mode of parasite nutrition which is somewhat ignored can help us to get some information which might lead the specialists in future to discover some drugs which can be used for treatment of inoperative cyst through their adherence to the biological materials that promote penetration of the drugs to the cyst (Rahdar *et al*., 2008).

This study proposed to determine the chemical components of hydatid cyst (fluid, protoscolices) of cysts isolated from liver and lung tissues of various intermediate hosts, in addition to infected and noninfected host tissues.

MATERIALS AND METHODS

Materials:- The present study include biochemical study on 38 fertile hydatid cysts isolated from the liver and Lungs of infected sheep, goats and cattle slaughtered at Duhok abattoir (Table 1) during the period between November 2009 to April 2010. In addition, 8 hydatid cyst fluids aspirated from humans during surgical removal of cysts from patients at Azadi Teaching Hospital in Duhok city during the period from March 2010 to July 2010. Samples were collected carefully and kept in cool box containing crushed ice and transported to Parasitology laboratory of College of Education, University of Zakho.

Table (1): The number of samples used in this study from different sources

Host	Liver	Lungs	Total
Sheep	5	5	10
Cattle	5	5	10
Goats	5	5	10
Human	4	4	8
Grand Total	19	19	38

Excision of the cyst from infected organs: The cyst was removed from the infected organ and transferred to a crystallizing basin, washed several times with physiological buffer solution (PBS) (pH 7.4) then opened and the cyst content was transferred to a clean container, then the sample was subjected to the following protocol.

Diagnosis of fertile cysts: - All cysts were checked by microscope (40X and 100X) to select the fertile cysts.

Digestion solution: - Was prepared by mixing equal volumes of H2So4 and HNo3 (Saeed and Al-Habbib, 1990). Used for digesting of tissues and protoscolices for determination of minerals.

Sample preparation:- For mineral determination, hydatid cyst fluid, protoscolices and host tissue samples were processed fluid as follow:

1- Hydatid fluid (HF): The HF of each sample was centrifuged at 4000 rpm for 20 minutes. Two ml of the supernatant was completed to 10 ml with deionized water. The sample was refrigerated until use.

2- Tissue and protoscolices: Three ml of digestion solution (1:1 H_2So_4 : HNo_3) was added to 1g of the tissue or protoscolices, left for 48 hr for complete digestion. The aliquot was diluted to 10 ml using deionized water, the sample was Millipore filtered and refrigerated until used. The studies parameters were determined using proper Biolabo-Reagents, France) according to manufacturer instructions.

Statistical analysis: One way analysis of variance (ANOVA) was used for statistical analysis and for comparison between the results (Cohen, 2003).

RESULTS

The level of Ions in hydatid cysts isolated from sheep liver and lungs along with both infected and non-infected sheep liver and lung tissues are shown in Table (2). It is obvious from the table that HFof cysts isolated from sheep liver contained the highest concentration of sodium which was 356±8.207 mg/dl followed by HF of lung cysts (336±6.454 mg/dl). While Na^+ concentration of protoscolices isolated from sheep liver and lung was much lower as compared with HF, and it is ranged from 188±7.427 to 171±5.842 mg/100g, respectively. However, the concentrations of Na^+ in infected and non-infected liver and lungs were intermediate as compared with hydatid cysts; furthermore, infected tissues (liver and lungs) showed higher sodium levels as compared with non-infected with the highest being in liver tissues (Table 2).

Table (2): Ions contents of hydatid cyst, infected and non-infected sheep liver and lung tissues (N: 5).

Parameters	Organ	Hydatid fluid (mg/dl)	Protosco-lice (mg/100g)	Infected tissue (mg/100g)	Non-infect-ed tissue (mg/100g)
Sodium	Liver	356 ± 8.207	188 ± 7.427	196 ± 7.461	160 ± 7.294
	Lung	336 ± 6.454	171 ± 5.842	178 ± 5.868	143 ± 5.736
Potassium	Liver*	39.84 ± 0.335	106 ± 0.894	83.66 ± 0.703	73.3 ± 0.618
	Lung*	36.85 ± 0.347	98.39 ± 0.926	77.39 ± 0.729	67.8 ± 0.64
Calcium	Liver	6.32 ± 0.056	6.824 ± 0.061	6.508 ± 0.057	6.954 ± 0.061
	Lung	6.206 ± 0.048	6.7 ± 0.052	6.39 ± 0.052	6.826 ± 0.054
Magnesium	Liver	0.778 ± 0.017	0.878 ± 0.02	0.858 ± 0.02	0.938 ± 0.02
	Lung	0.792 ± 0.013	0.896 ± 0.015	0.87 ± 0.014	0.952 ± 0.015
Iron	Liver*	0.088 ± 0.003	0.0836 ± 0.003	0.2288 ± 0.008	0.2816 ± 0.01
	Lung*	0.0786 ± 0.002	0.0747 ± 0.002	0.2044 ± 0.004	0.2515 ± 0.006

Protoscolices of cysts isolated from sheep liver and lungs showed the highest concentration of potassium which was greater by about 2.7 folds as compared with HF of the same cysts. Regarding host tissues, both infected liver and lungs tissues showed higher potassium concentration as compared with non-infected tissue with the highest being in lung tissues (Table 2). With respect to Calcium and Magnesium ions, their concentrations were almost the same in hydatid cysts, infected and non-infected liver and lung tissues as indicated in Table (2).

Very little amount of iron was detected in HF and protoscolices of both liver and lungs cysts. On the other hand, infected and non-infected liver and lung tissues showed higher concentration of iron as compared with hydatid cysts and this difference was statistically significant ($P \leq 0.05$). In conclusion, there was a wide variation in the concentration of the four different ions in the protoscolices and HF. Sodium was higher in HF, whereas, K^+ level were higher in protoscolices.

The level of Ions in hydatid cysts isolated from goats liver and lungs along with both infected and non-infected goats liver and lung tissues are shown in Table (3). It is obvious from the table that HF of cysts isolated from goats liver contained the highest concentration of sodium which was 220±7.576 mg/dl followed by HF of lung cysts (202±5.958 mg/dl). While, Na^+ concentration of protoscolices isolated from goats liver and lung was much lower as compared with HF, and it is ranged from 66.07±6.856 to 49.85±5.391 mg/100g, respectively.

However, the concentration of Na^+ in infected and non-infected tissues was much lower as compared with hydatid cysts (Table 3). But infected tissues (both liver and lungs) showed higher Na^+ levels than non-infected tissues. Generally Na^+ levels were higher in liver cyst, infected and non-infected tissues as compared with correspondent lung cysts and tissues.

Table (3): Ions contents of hydatid cyst, infected and non-infected goats liver and lung tissues (N: 5).

Parameters	Organ	Hydatid fluid (mg/dl)	Protosco-lice (mg/100g)	Infected tissue (mg/100g)	Non-infect-ed tissue (mg/100g)
Sodium	Liver	220 ± 7.576	66.07 ± 6.856	72.75 ± 6.887	39.95 ± 6.734
	Lung	202 ± 5.958	49.85 ± 5.391	56.46 ± 5.416	24.02 ± 5.296
Potassium	Liver*	36.65 ± 0.309	97.86 ± 0.823	76.97 ± 0.647	67.44 ± 0.567
	Lung*	33.9 ± 0.318	90.52 ± 0.853	71.19 ± 0.67	62.38 ± 0.588
Calcium	Liver	8.534 ± 0.076	9.212 ± 0.081	8.788 ± 0.076	9.384 ± 0.083
	Lung	8.374 ± 0.067	9.044 ± 0.072	8.63 ± 0.069	9.214 ± 0.072
Magnesium	Liver	0.678 ± 0.016	0.766 ± 0.016	0.746 ± 0.016	0.814 ± 0.018
	Lung	0.692 ± 0.01	0.778 ± 0.012	0.758 ± 0.012	0.828 ± 0.012
Iron	Liver*	0.083 ± 0.003	0.0789 ± 0.003	0.2158 ± 0.008	0.266 ± 0.01
	Lung*	0.0736 ± 0.002	0.0699 ± 0.002	0.1914 ± 0.004	0.236 ± 0.006

Protoscolices of cysts isolated from goats liver and lungs showed the highest concentration of potassium which was about 2.65 folds as compared with HF of the same cysts. Regarding host tissues, both infected liver and lungs tissues showed higher potassium concentration as compared with non-infected tissue (Table 3), and these differences were statistically significant ($P \leq 0.05$).

With respect to Calcium and Magnesium, their concentrations were almost the same in hydatid cysts, infected and non-infected host tissues as indicated in Table (3).

Very small amount of iron was detected in HF and protoscolices of both liver and lungs cysts. On the other hand, infected and non-infected liver and lung tissues showed higher concentrations of iron as compared with hydatid cysts and this difference was statistically significant ($P \leq 0.05$).

The level of Ions in hydatid cysts isolated from cattle liver and lungs along with both infected and non-infected cattle liver and lung tissues are shown in Table (4). HF of cysts isolated from cattle liver contained the highest concentration of sodium which was 238±7.659 mg/dl followed by HF of lung cysts (220±6.024 mg/dl). While Na^+ concentration of protoscolices isolated from cattle liver and lung was much lower as compared with HF, and it is ranged from 82.39±6.931 to 66±5.45 mg/100g, respectively. However, the concentration of Na^+ in infected and non-infected tissues was intermediate as compared with hydatid cysts (Table 4). Liver cysts and infected liver tissues showed higher Na^+ levels.

Table (4): Ions contents of hydatid cyst, infected and non-infected cattle liver and lung tissues (N: 5).

Parameters	Organ	Hydatid fluid (mg/dl)	Protosco-lice (mg/100g)	Infected tissue (mg/100g)	Noninfect-ed tissue (mg/100g)
Sodium	Liver	238 ± 7.66	82.39 ± 6.931	89.14 ± 6.963	55.99 ± 6.808
	Lung	220 ± 6.024	66 ± 5.45	72.67 ± 5.475	39.88 ± 5.354
Potassium	Liver*	32.67 ± 0.274	87.22 ± 0.732	68.6 ± 0.578	60.11 ± 0.506
	Lung*	30.22 ± 0.284	80.68 ± 0.759	63.46 ± 0.597	55.6 ± 0.523
Calcium	Liver	6.954 ± 0.061	7.51 ± 0.07	7.158 ± 0.063	7.648 ± 0.066
	Lung	6.826 ± 0.054	7.37 ± 0.06	7.03 ± 0.055	7.51 ± 0.06
Magnesium	Liver	0.71 ± 0.016	0.80 ± 0.018	0.78 ± 0.016	0.85 ± 0.018
	Lung	0.722 ± 0.01	0.816 ± 0.012	0.794 ± 0.012	0.866 ± 0.012
Iron	Liver*	0.088 ± 0.003	0.084 ± 0.003	0.23 ± 0.008	0.282 ± 0.009
	Lung*	0.08 ± 0.001	0.076 ± 0.001	0.209 ± 0.003	0.257 ± 0.004

Protoscolices of cysts isolated from cattle liver and lungs showed the highest concentration of potassium which was about 2.65 folds as compared with hydatid fluid of the same cysts. Regarding host tissues, both infected liver and lungs tissues showed higher potassium concentration as compared with non-infected tissue, with the highest levels being in liver cyst and tissues (Table 4).

With respect to Calcium and Magnesium, their concentrations were almost the same in hydatid cysts, infected and non-infected host tissues as indicated in Table (4).

Very small amount of iron was detected in HF and protoscolices of both liver and lungs cysts. On the other hand, infected and non-infected liver and lung tissues showed higher concentration of iron as compared with hydatid cysts and this difference was statistically significant ($P \leq 0.05$). Regarding tissues, liver tissues showed slightly higher Fe^{++} levels than lung tissues.

The level of Ions in HF of cysts removed from human liver and lungs are shown in Table (5). It is obvious from the table that HF of liver cysts contained the highest concentration of sodium which was 191±7.768 mg/dl followed by HF of lung cysts (180±7.02 mg/dl).

The HF of cysts removed from human liver contained the highest concentration of potassium which was 31.76 ± 0.153 mg/dl, followed by HF of lung cysts (29.44 ± 0.353 mg/dl). This deference was statically significant ($P \leq 0.05$).

Table (5): Ions contents of hydatid fluid of cysts removed from human (mg/dl) (N: 4).

Parameters	Liver	Lung	Normal values
Sodium	191 ± 7.768	180 ± 7.02	92-500
Potassium	31.76 ± 0.153	29.44 ± 0.353	98-470
Calcium	7.943 ± 0.073	7.76 ± 0.08	100-300
Magnesium	0.74 ± 0.015	0.763 ± 0.02	68.02-97.50
Iron	0.09 ± 0.003	0.078 ± 0.002	0.045-0.16

With respect to Calcium and Magnesium, their concentrations were almost the same in HF of both liver and lung cysts as indicated in Table (5).

Very small amount of iron was detected in HF of both liver and lung cysts which was slightly higher in liver cyst HF, and deference was statically non-significant (P> 0.05).

Table (6) shows comparison between the ion contents of hydatid cysts isolated from liver of various intermediate hosts. Regarding the Ions, there are some differences in their levels in HF and protoscolices of different intermediate hosts. HF of all hosts contained the higher levels of Na^{+} than protoscolices, with the highest being in HF of sheep cysts (356 ± 8.207 mg/dl). On the other hand, protoscolices contained higher levels of K^{+} ions, also the highest being in sheep cysts.

Table (6): Comparison between ion contents of hydatid cysts isolated from livers of different hosts.

Parameters	Sheep (N:5)		Goat (N:5)		Cattle (N:5)		Human N:4)
	Hydatid fluid (mg/dl)	Protos-colices (mg/100g)	Hydatid Fluid (mg/dl)	Protos-colices (mg/100g)	Hydatid Fluid (mg/dl)	Protos-colices (mg/100g)	Hydatid Fluid (mg/dl)
Sodium	356 ± 8.207	188 ± 7.427	220 ± 7.576	66.1 ± 6.856	238 ± 7.659	82.39 ± 6.931	191 ± 7.768
Potassium	39.8 ± 0.335	106 ± 0.894	36.7 ± 0.309	97.86 ± 0.823	32.67 ± 0.274	87.22 ± 0.732	31.8 ± 0.153
Calcium	6.32 ± 0.056	6.82 ± 0.061	8.53 ± 0.076	9.21 ± 0.081	6.954 ± 0.061	7.508 ± 0.066	7.943 ± 0.073
Magnesium	0.78 ± 0.017	0.88 ± 0.02	0.68 ± 0.016	0.77 ± 0.016	0.71 ± 0.016	0.802 ± 0.018	0.74 ± 0.015
Iron	0.09 ± 0.003	0.08 ± 0.003	0.08 ± 0.003	0.079 ± 0.003	0.088 ± 0.003	0.084 ± 0.003	0.09 ± 0.003

With respect to Ca^{++}, almost there was no difference in its level in HF and protoscolices, regarding hosts, the highest level observed in goats HF and protoscolices, while the lowest level was in sheep. Both HF and protoscolices in all hosts contain low levels of Mg^{++} (Table 6).

Small quantities of iron were detected in hydatid cyst which was slightly higher in liver cysts as compared with lung cysts (Tables 6 and 7).

Table (7): Comparison between ion contents of hydatid cysts isolated from lungs of different hosts.

Parameters	Sheep (N:5)		Goat (N:5)		Cattle (N:5)		Human N:4)
	Hydatid fluid (mg/dl)	Protos-colices (mg/100g)	Hydatid Fluid (mg/dl)	Protos-colices (mg/100g)	Hydatid Fluid (mg/dl)	Protos-colices (mg/100g)	Hydatid Fluid (mg/dl)
Sodium	336 ± 6.454	171 ± 5.842	202 ± 5.96	49.85 ± 5.391	220 ± 6.024	66 ± 5.45	180 ± 7.02
Potassium	36.85 ± 0.347	98.4 ± 0.926	33.9 ± 0.318	90.52 ± 0.853	30.2 ± 0.284	80.68 ± 0.759	29.44 ± 0.353
Calcium	6.206 ± 0.048	6.7 ± 0.052	8.37 ± 0.067	9.044 ± 0.072	6.83 ± 0.054	7.37 ± 0.06	7.76 ± 0.08
Magnesium	0.79 ± 0.013	0.9 ± 0.015	0.69 ± 0.01	0.778 ± 0.012	0.72 ± 0.01	0.82 ± 0.012	0.763 ± 0.02
Iron	0.079 ± 0.002	0.075 ± 0.002	0.074 ± 0.002	0.07 ± 0.002	0.08 ± 0.001	0.076 ± 0.001	0.078 ± 0.002

DISCUSSION

Differences in the metabolism of hydatid cyst from different intermediate hosts such as sheep, goats, cattle camels and human is most probably due to complex geographical strains as well as their biochemical and physiological differences (Frayha and Haddad, 1980; McManus, 1981; and Refik, *et al*., 2002). Thus, wide variations in the concentrations of ions and other chemicals have been reported by Frayha and Haddad (1980). Since Na^+ is the major extracellular cation, the highest Na^+ concentrations was observed in HF, while, comparatively, a lower Na^+ concentration was exhibited by protoscolices. On the other hand, since K^+ is the major intracellular cation, highest K^+ concentration was observed in protoscolices which is about 2.7 folds as compared with HF. High cation concentration in protoscolices may be due to the presence of cation-rich calcareous corpuscles present in the tissue (Frayha and Haddad, 1980).

Also high NaCl concentration is the characteristics of the body fluid of ecto- and endoparasites (Von, 1979) as well as the hydatid cyst fluid (Frayha and Haddad, 1980).

The concentration of Ca^{++} was much lower than Na^+ and K^+ ions. Furthermore, Ca^{++} concentration in HF and protoscolices in goats was slightly higher as compared with others, and the lowest was exhibited by cattle. Radfar and Iranyar (2004) also reported such a low level of Ca^{++} in the HF of sheep, goats, cattle and human cysts. The presence of Ca^{++} in the hydatid cyst fluid is of vital importance since it prevents the hydatid cyst fluid acidity and its accumulation as calcareous body in the cyst (Rahdar *et al*., 2008).

The concentration of Mg^{++} was much lowers than Ca^{++} ion concentration. Since Mg^{++} has narcotics action on the body activities, it is usually excreted to the exterior via conventional transport mechanisms (Bernath *et al*. 1985).

However, in the current study, sheep, goats and cattle hydatid cyst, showed comparatively slightly higher Ca^{++} and Mg^{++} levels in protoscolices than hydatid cyst fluid. This agrees with the results of Frayha and Haddad, (1980) who indicated that the amount of Ca^{++} and Mg^{++} in protoscolices were more than that of hydatid fluid.

Since there are no detailed systematic studies on comparative biochemical composition in hydatid cysts from different hosts (sheep, goats, cattle, camel and human), it is very difficult to compare the results. However, there is a general tendency of cations of physiological importance (Na^+ and K^+) to exhibit the highest levels in sheep among the studied animals. On the other hand, the lowest levels of Na^+ and K^+ were observed in human hydatid cyst fluid.

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الخلاصة

تضمنت هذه الدراسة مقارنة بين المكونات الكيموحياتية للاكياس المائية (السائل العدري والرؤوس البدائية) إضافة الى الانسجة المصابة وغير المصابة المعزولة من كبد ورئتي الاغنام والماعز والماشيه المذبوحة في مجزرة دهوك للفترة من تشرين الثاني ٢٠٠٩ الى نيسان ٢٠١٠. إظافة الى عينات سائل الاكياس المائية المستأصلة من المصابين في مستشفى آزادي التعليمي في دهوك للفترة من آذار ٢٠١٠ الى تموز ٢٠١٠. وقد تمت دراسة مستوى الآيونات (Na^+, K^+, Ca^{++}, and Mg^{++}) والحديد Fe^{++} في الاكياس المائية والانسجة المصابة للمضائف المختلفة.

من بين الآيونات أظهر آيون الصوديوم Na^+ مستويات عالية في السوائل العدرية للاكياس المأخوذة من مختلف المضائف مع ارتفاع ملحوظ في اكياس اكباد الاغنام المصابة (٣٥٦±٨،٢٠٧ ملغم/ديسيلتر). كذلك فإن الانسجة المصابة أظهرت مستويات عالية من آيون الصوديوم مع ارتفاع ملحوظ في اكياس اكباد ورئات الاغنام المصابة (١٩٦±٧،٤٦١ ملغم/ديسيلتر و ١٧٨±٥،٨٦٨ ملغم/ديسيلتر على التوالي).

الرؤوس البدائيةلاكياس الاكباد والرئات المصابة أظهرت مستويات عالية من آيون البوتاسيوم +K . ومن بين الانسجة، اظهرت الانسجة المصابة مستويات عالية منه مع ارتفاع ملحوظ في الرئات (من: ٠،٥٩٧±٦٣،٤٦ ملغم/١٠٠غم الى: ٠،٧٢٩±٧٧،٣٩ ملغم/١٠٠غم).

وجدت مستويات عالية تقريبا من آيون الكالسيوم ++Ca للسائل العدري والرؤوس البدائية للاكياس المائية مع ارتفاع ملحوظ في السائل العدري للاكياس المستاصلة من انسجة الماعز (كبد: ٠،٠٦١±٦،٩٥٤ ملغم/ديسيلتر, رئات: ٠،٠٥٤±٦،٨٢٦ ملغم/ديسيلتر). أما بالنسبة لمستوى المغنسيوم ++Mg، فكان واطئا في اكياس وانسجة جميع المضائف (من: ٠،٠١٦±٠،٧٤٦ ملغم/١٠٠غم الى: ٠،٠١٥±٠،٩٥٢ ملغم/١٠٠غم).

كميات قليلة من الحديد ++Fe وجدت في اكياس جميع المضائف، مع ارتفاع طفيف في اكباد الماشية (السائل العدري: ٠،٠٠٣±٠،٠٨٨٢ ملغم/ديسيلتر والرؤوس البدائية:٠،٠٠٣±٠،٠٨٣٨ ملغم/١٠٠غم ونسيج الاكباد المصابة: ٠،٠٠٨±٠،٢٢٩٣ ملغم/١٠٠غم).

پوختە

دڤێ ڤەکولینێ دا، بەراوەردکرەنکا بایوکیمیایی هاتە کرن دناڤبەرا کیسکێت ئاڤێ (ئاڤا کیسکا و سەرکێت دەستپێکی) زێدەباری پوشەکێت ئێشگرتی و پێت ساخلەم پێت مێلاك وپشێت پەز وبزن وچێلێت هاتینە سەر ژێکرن ل سەرژێگەها دهوك دناڤبەرا چریا دووێ ٢٠٠٩ هەتا نیسان ٢٠١٠. هەروەسا چەند نمونەك ژ کیسکێت ئاڤێ پێت کو هاتینە ژێڤەکرن ژ نەخوشێت نەخوشخانا ئازادی یا فێرکرنێ دناڤبەرا ئادار٢٠١٠ الی تیرمەھ ٢٠١٠. ودڤێ ڤەکولینێ دا ئاستێت ئایونێت (Na^+, K^+, Ca^{++}, and Mg^{++}) وئاسنی Fe^{++} دکیسکێت ئاڤێ و پوشەکێت ئێشگرتی پێت خانەخوییێت جورەوجور هاتنە خواندن.

دناڤ ئایونا دا، ئایونێ صودیومی Na^+ ئاستەکێ بلند دیار کر د ئاڤا کیسکێت وەرگرتی دگەل بلندیەکا بەرچاڤ دناڤ کیسکێت مێلاکێت پەزێت ئێشگرتی (٣٥٦±٨,٢٠٧ ملغم/دیسیلتر). هەروەسا پوشەکێت ئێشگرتی ئاستێت بلند دیار کرن ژ ئایونێ صودیومی دگەل بلندیەکا بەرچاڤ د کیسکێت مێلاك وپشێت پەزێت ئێشگرتی (١٩٦±٧,٤٦١ ملغم/دیسیلتر و ١٧٨±٥,٨٦٨ ملغم/دیسیلتر د دویف ئێك دا).

سەرکێت دەستپێکی پێت مێلاك وپشێت ئێشگرتی ئاستەکێ بلند دیار کر ژ ئایونێ پوتاسیومی K^+ . ودناڤ پوشەکا دا، پوشەکێت ئێشگرتی ئاستەکێ بلند دیار کر دگەل بلندیەکا بەرچاڤ دناڤ پشێ دا (ژ: ٠,٥٩٧±٦٣,٤٦ ملغم/١٠٠غم تا: ٠,٧٢٩±٧٧,٣٩ ملغم/١٠٠غم).

ئاستێت بلند ژ ئایونێ کالسیومی Ca^{++} دناڤ ئاڤ وسەرکێت دەستپێکی پێت کیسکێت ئاڤێ دگەل بلندیەکا بەرچاڤ د ئاڤا کیسکێت ژ پوشەکێت بزنا وەرگرتین (مێلاك: ٠,٠٦١±٦,٩٥٤ ملغم/دیسیلتر, پش: ٠,٠٥٤±٦,٨٢٦ ملغم/دیسیلتر). و سەبارەت ئایونێ مگنسیومی Mg^{++}، یێ نزم بو د کیسك وپوشەکێت هەمی خانەخویا دا (ژ: ٠,٠١٦±٠,٧٤٦ ملغم/١٠٠غم تا: ٠,٠١٥±٠,٩٥٢ ملغم/١٠٠غم).

هندەك قەباریت کیم ژ ئاسنی Fe^{++} هاتنە دیتن دناڤ کیسکێت هەمی خانەخویا دا دگەل بلندیەکا کیم دمێلاکێت چێلا دا (ئاڤ: ٠,٠٠٣±٠,٠٨٨٢ ملغم/دیسیلتر سەرکێت دەستپێکی:٠,٠٠٣±٠,٠٨٣٨ ملغم/١٠٠غم وپوشەکێ مێلاکێت ئێشگرتی: ٠,٠٠٨±٠,٢٢٩٣ ملغم/١٠٠غم).

PREVALENCE OF HYDATID DISEASE AMONG SLAUGHTERED ANIMALS IN SLEMANI PROVINCE/ KURDISTAN-IRAQ.

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Abstract

Hydatid cyst (HC) is one of the common silent zoonotic disease worldwide distributions, with medical importance and economical effect. The present study was conducted from January 2011 to March, 2012 to determine the prevalence rate of hydatid disease among slaughtered livestock including sheep, goats and cattle in Slemani province. A total of 7698 slaughtered animals were examined from different abattoirs for hydatid cyst during post mortem inspection including 6550 sheep, 348 goats and 800 cattle. The study showed that the prevalence rates among slaughtered animals were 12.7% in sheep, 4.8% in goats and 4.3% in cattle. The fertility rate of HCs was higher in sheep than that of goats and cattle furthermore, the rate of the fertility was higher in liver cysts than in lung cysts in all studied intermediate hosts.

KEYWORD: *Hydatid cyst, E.granulosus, prevalence, Hydatid Disease*

Introduction

Echinococcosis or hydatid Disease is one of the neglected silent helminthes infection caused by the larval stages of cestode belonging to the genus Echinococcus (family Taeniidae), which is a major public health problem in developing countries (Siracusano *et al*., 2012). The larval infection is characterized by long-term growth of the metacestode in the intermediate host (Zhang *et al*., 2003).

Hydatid disease has a dual impact on human health and livestock production. Human populations dependence on livestock are not only most at direct risk from zoonotic disease, but are most vulnerable to the indirect impacts on health, or reduced production on livelihoods and food security, which exacerbates the poverty cycle (Molyneux, *et al*., 2011). Humans become infected accidentally by ingesting food or water contaminated with fecal material containing *E. granulosus* eggs passed from infected carnivores, or when they handle pet or infected dogs (WHO, 2006; Satoskar, *et al*., 2009). The commonest sites of infection are the liver and lungs (Markell *et al*., 1999), whereas, it was noticed that the disease cannot be transmitted between humans or from human to dogs (Rood and Kelly, 2009).

There are six species of *Echinococcus*: *E. granulosus, E. multilocularis, E. vogeli, E. oligarthrus, E. shiquicus and E. felidis* (Brunette, 2012), two of these species are of medical importance, which are *E. granulosus* and *E. multilocularis* causing Cystic Echinococcosis (CE) and Alveolar Echinococcosis (AE), respectively. This study aimed to determine the status and analysis of epidemiological factors of hydatid disease among slaughtered domestic animals in Slemani province.

Materials and methods:

A total of 7698 slaughtered animals of both sexes were examined for hydatid cyst during post mortem inspection including 6550 sheep, 348 goats and 800 cattle. Livers and lungs were inspected by cutting, and both surfaces were examined by incisions as well as examined through visual inspection. The Hydatid cyst fluid (HCF) was collected from individual cyst, washed by phosphate buffer saline (PBS) and checked for the presence of protoscolecies (Latif *et al*., 2010).

The investigation of hydatid cysts was carried out for the following parameters: location of cysts (organ specificity), cyst fertility and viability of protoscolices.

Animal intact cysts were obtained from Slemani abattoir, which included 10 individual cysts from goat, 12 from cattle and 20cysts from sheep. The samples were transported to the laboratory in a cool box. Individual cysts were grossly investigated for degeneration and calcification (WHO, 2003). Then according to the animal species, size of cyst and infected organs around 10% of hydatid cysts were randomly selected for fertility and viability study.

Fertility and Viability

After aspiration of cyst fluid, the fluid was centrifuged, then one drop of precipitated was taken by Pasteur pipette and examined by light microscope under (40x) for the presence of protoscolecies. The cysts which were without protoscolices were considered as non fertile (Daryani *et al*., 2006).

Eosin dye (vital stain) was used for determination of viability of protoscolices (Daryani *et al*., 2006).The protoscolex with inactive flame cell or stained with eosin considered as dead (Esfahani and Youssefi, 2010).

Statical analysis

The data were analyzed using chi-squire (GraphPad Prism 6).

Results

The result revealed that the prevalence rate of Echinococcosis was significantly differences ($p<0.05$) between all animal species (Table1). The prevalence rate of HC in sheep, goats and cattle was 12.7%, 4.8% and 4.3%, respectively. The highest rate of infection was observed in sheep followed by goats and cattle.

The results of the present study showed that the sex of the slaughtered animals has non significant effect on the distribution of HC, as the rate of HC in males and females were very close (Table 1).

Table (1): The prevalence rate of HCs among different slaughtered animals in Slemani province.

Species	No. of inspected animals			No. of Infected		Rate of infection %		Total infected	
	Male	Female	Total	Male	Female	Male	Female	No.	%
Sheep	2400	4150	6550	313	522	13.04	12.57	835	12.7
Goats	227	121	348	11	6	4.8	4.9	17	4.8
Cattle	700	100	800	31	4	4.42	4	35	4.3
Total	3327	4371	7698	355	532	10.67	12.17	887	11.52

Sex: $X^2 = 3.32$ $p = 0.68$ df=1
Species: $X^2 = 54.24$ $p = 0.0002$ df=2

Organ specificity

The distribution of HCs in various organs among slaughtered animals is shown in Table (2). The present study revealed that the co-infection of the liver and lungs was the predominant infection and the preponderant site of hydatid cyst was the liver followed by lungs, this indicates that the liver is the primary site for cyst development and lung involvement comes as a secondary consequence.

Table (2): Distribution of hydatid cysts in the internal organs among slaughtered animals in Slemani province.

Species	No. of inspected animal	No. and % of infection		Infected organs					
				Liver		Lung		Liver and Lung	
		No.	%	No.	%	No.	%	No.	%
Sheep	6550	835	12.75	79	9.46	44	5.26	712	85.26
Goats	348	17	4.89	7	41	3	17.6	7	41.1
Cattle	800	35	4.38	14	40	5	14.2	16	45.7
Total	7698	887	11.52	100	11.2	52	5.8	735	82.8

$X^2 = 60.2$ $p= 0.0001$ df=2

Type of cysts

The nature of the isolated cysts of slaughtered animals was studied on the base of fertile, sterile and calcified (Table 3). In sheep, the rates of fertile, sterile and calcified cysts among 100 examined cysts were 86, 9and 5%, respectively. In goats, the rates of fertile, sterile and calcified cysts among 40 examined cysts were 60, 20 and 20%, respectively. The same pattern was observed in cattle, 58, 22 and 20%, respectively.

Table (3): The number and percentage of cyst types in different intermediate hosts

Intermediate host	No. of cysts	Type of cysts					
		Fertile		Sterile		Calcified	
		No.	%	No.	%	No.	%
Sheep	100	86	86	9	9	5	5
Goats	40	24	60	8	20	8	20
Cattle	50	29	58	11	22	10	20
Human	12	10	83.3	1	8.3	1	8.3
Total	202	149	73.7	29	14.3	24	11.8

X^2= 19.22 p=0. 003 df=6

Discussion

The highest rate of infection was observed in sheep followed by goats and cattle. Similar high rate of infection in sheep have been reported by Amin (2007) in Slemani, Al-Berwari (2012) and Abdullah and Mero(2013) in Duhok, they suggested that due to the host specificity and strain distribution in this region, in addition in Kurdistan sheep are more desirable for rearing due to their consumption preference to fulfill religious and social requirements and they are more adapted with dog than goats or cattle also their feeding habit have effect on the infection rate.

On the other hand, lower rates were reported by Bajalan (2006) in Kalar; Kadir and Rasheed, (2008) in Kirkuk, and Jarjees and Al-Bakri, (2012) in Mosul. The low prevalence rate in goats, in the present study may be due to feeding habit of this animal, as they eat the higher parts of herbage that are exposed to the sunlight which decrease the viability of the eggs (Torgerson and Budke, 2003), also the low prevalence rate of HC among cattle in the present study may be due to rearing them in cowshed with better care which relatively has no contact with the source of infection (Thompson and McManus, 2002).

In contrast Meerkhan and Abdullah, (2012) in Duhok, reported higher infection rates among cattle than in sheep and they attributed it to the fact that cattle are slaughtered at older age which increase the risk of exposure to eggs of *E. granulosus* and cattle eat larger amount of herbage than sheep and goats.

This difference in prevalence rate among livestock attributed, to the mode of grazing, presence of the definitive host (carnivore) and other environmental factors. El-Ibrahim (2009), and the strains of the parasite also have essential role in HC distribution which is known as host specificity (Hama *et al*., 2012).

The results of the present study revealed that the sex of the slaughtered animals has no effect on the prevalence rate of HCs, as the rate of HC in males and females were very close. This finding is in agreement with Rokni (2009) and Salem *et al*. (2011) they stated that both sex has the same chance to get infection which usually depend on the contact with the source of infection and habit of grazing. In contrast Ibrahim (2010) and Muqbil *et al*. (2012) reported that females of sheep and goats were more likely to have HC infection than males as males were slaughtered in younger age while female sheep and goats were usually maintained for longer periods than males to give offspring several times before slaughtering.

Organ specificity

The present study revealed that the co-infection of the liver and lungs was the predominant infection and the preponderant site

of hydatid cyst was the liver followed by lung, this indicates that the liver is the primary site for cyst development and lung involvement comes as a secondary consequence. Similar findings were reported by many researchers from different parts of the world Saida and Nuraddin (2011) in Erbil; Muqbil *et al*. (2012) in Baghdad; Ioan *et al*. (2012) in Romania, and Jarjees and Al-Bakri (2012) in Mosul they stated that the liver acts as the first barrier for the oncosphere penetrating the intestinal mucosa to reach the portal vein and carried by the blood stream to all parts of the body, due to the large size of oncosphere, most of them become settled in the liver. On the other hand, the results of this study did not coincide with those of some other workers in which they found that the lungs were the most predominant site for Echinococcosis Abdullah, (2010) in Duhok and Lotfi *et al*. (2010) in Iran they attributed it to the larger lymphatic vessels which provides a chance for the oncosphere to reach lymphatic lacteal then travel through the lymph to the lungs before being translocated in venules to reach the liver, or the oncosphere may be released from the egg during rumination which may gain access to the lung.

Type of cysts

In the present study the majority of cysts in sheep (86%) and goats (60%) were fertile, while the fertility rate of HCs in cattle was lower (58%) than in sheep and goats, this indicate that sheep and goats have an essential role for the perpetuation of the life cycle then spread of the disease.

These results are consistent with Ioan *et al*. (2012) in Romania and Jarjees and Al-Bakri (2012) in Iraq. The fertility rate of hydatid cyst is an important factor in the epidemiological studies, due to the possibility of fertile cysts to disseminate the disease and to determine the main species as a potential host in the spread of the infection (Mahmoud, 1980). The variation in fertility rate among different intermediate hosts due to the difference in strain of *E. granulosus* (Hama *et al*. 2013).

In contrast Saeed *et al*. (2000) and Fikire *et al*. (2012) found that the fertility rate was higher in the lung cysts than in liver cysts and they attributed it to the relatively softer consistency of lung tissue which allows the easier development of the cyst. The fertility of hydatid cysts varies depending on the host species (Saeed *et al*., 2000). The variation in the prevalence rate of fertile cysts in different intermediate hosts in the present study may be due to the presence of different strains of *E. granulosus* which might cause the variation in the fertility rate in various environmental regions (McManus, 2006).

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ڕێژەی بڵاو بونەوەی نەخۆشی تورەکەی ئاوی (Hydatid cyst) لە نێو ئاژەڵە سەربڕاوەکان لە پارێزگای سلێمانی/ کوردستان-عراق

تورەکەی ئاوی یەکێکە لە نەخۆشیە بێدەنگە شاراوەکان و لە نەخۆشیە سەرچاوە ئاژەڵیە کانە، وە لەسەرتاسەری جیهاندا بەربڵاوە و کاریگەری هەیە لەسەر تەندروستی و وە ئابوری کۆمەڵگا، ئەم تویژینەوەیە ئەنجام درا لە ماوەی کانونی یەکەم ٢٠١١ تا ئازاری ٢٠١٢ بۆ دیاری کردنی ڕێژەی بڵاو بونەوەی نەخۆشی تورەکەی ئاوی لە نێوان ئاژەڵە سەربڕاوەکاندا (مەڕ، بزن وە ڕەشەوڵأخ) لە پارێزگای سلێمانی وە پشکنین بۆ ٧٦٩٨ سەر ئاژەڵ کرا پاش سەربڕین بۆ زانینی توش بوون بە نەخۆشی تورەکەی ئاوی لە کوشتارگە جیا جیا کانی پارێزگای سلیمانی کە بریتی بوون لە ٦٥٥٠ سەر مەڕ، ٣٤٨ سەر بزن وە ٨٠٠ سەر ڕەشە وڵأخ. ڕێژەی تووشبوون بریتی بوو لە ١٢,٧% لەمەڕ دا، ٤,٨% لە بزن دا وە ٤,٣% لە ڕەشەوڵاخ دا. تێکڕای ڕێژەی بەپیتی لەو تورەکە ئاویانەی کە لە مەڕ وە وەرگیرابوون زیاتربوو لەوانەی کە لە بزن و ڕەشەوڵاخەوە وەرگیرابون هەروەها ئەوەش زانرا کە ئەم ڕێژەیە لەو تورەکانەی کە لە جگەرەوە وەرگیراون زیاترە لەوانەی کە لە سیەکانەوە وەرگیراون لە هەموو ئاژەڵەکاندا.

نسبة انتشار داءالاكياس المائية (Hydatid cyst) بين الحيوانات المذبوحة في محافظة السليمانية/ كوردستان– العراق

الملخص:

داء الاكياس المائية هو احد الامراض المخفية ذو المنشأ الحيواني وله انتشار عالمي, وذو تأثير طبي واقتصادي في المجتمع. أجريت هذة الدراسة خلال الفترة من كانون الثاني٢٠١١ الى اذار٢٠١٢ لتحديد نسبة انتشار داءالاكياس المائية بين الحيوانات المذبوحة والتي تضمنت الاغنام والماعز والمواشي في محافظة السليمانية.وتم فحص و التحري ل ٧٦٩٨ حيوان تم ذبحهم في مختلف المجازر للتحري عن داءالاكياس المائية و شملت ٦٥٥٠ راسا من الاغنام و٣٤٨ ماعز و ٨٠٠ من المواشي . بلغت نسبة الانتشار لهذا الداء١٢,٧% في الاغنام و ٤,٨% في الماعز و ٤,٣% في المواشي. وجد اعلى معدل لنسبة الخصوبة للاكياس المائية في الاغنام والتي كانت اعلى مما هي عليه في الماعز و المواشي وأيضا لوحظ ان معدل الخصوبة كان أكثر في الاكياس المعزولة من الكبد مقارنة بالاكياس المعزولة من الرئة في جميع الحيوانات التي تمت دراستها.

HISTOPATHOLOGICAL STUDY OF SOME ORGANS AFTER LONG-TERM TREATMENT WITH DEXAMETHASONE IN MALE RABBITS.

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Abstract:

The purpose of the study was to determined the histopathological changes in the adrenal gland, Kidney and liver which results from treatment with dexamethasone(DEX) .Thirty local male rabbits in Basra city were divided into three groups,the first group control group was given the normal saline orally , group two were orally given DEX. (0.2 mg/ k g/ BW) and the third group was treated with oral administration of DEX.at a dose of (1 mg. / k g/ BW) for (60) days. The results showed several histopathological changes in the adrenal gland of rabbits in both treated groups represented by congestion of blood vessels, degeneration, vaculation and necrosis of cells in the zona glomerulosa and zona fasciculata of the adrenal cortex. In treated rabbits,the histological section of kidney show some changes represented by degeneration, edema and cells necrosis of epithelial lining of convoluted tubules, infiltration of inflammatory cells and atrophy of some glomeruli. Liver of treated rabbits show ballooning hepatocytes and extension the sinuses.

Keywords: Dexamethasone, histopathology, adrenal gland , Kidney , liver

Introduction

Adrenocortical steroids are used in medicine for their anti-inflammatory and immune suppressive effects such as severe asthma, acquired hemolytic anemia, severe allergic reactions of all kinds and organ transplant rejection (Katzung, 2007).

Dexamethasone(DEX) synthetic glucocorticoid with potent anti inflammatory activity, (Gyton and Hall, 2004). The potency of its anti inflammatory activity has been quantified to be (25-30) times compared with hydrocortisone (Ganong, 2003).

Dexamethasone is used in adrenocortical suppression which lead to the increase of lipid droplets in the zona fasiculata and zona glomerulosa in the cortex of gland (Hemmaid, 2009). In the adult human and animal models, glucocorticoids such as DEX have been associated with marked reduction in bone mineralization (Van.1994) by reducing intestinal calcium absorption and reducing renal calcium reabsorption (Adachi and Papiaoannou, 2001). Dexamethasone stimulate mobilization of free fatty acids from adipose tissue and could also contribute to the development of hepatic lipidosis (House and Hill, 2002). Long term treatment with DEX have many side effect like increasing cholesterol, triglycerides and fatty acid concentration in the blood (Serverino *et al.*,2002).

Aim of study

Study the Histopathological changes in Adrenal gland, Kidney and Liver. after oral administration of Dexamethasone for (60 day).

Material and Methods

In this study, thirty (30) male rabbits weighting (1000 -1300) g. (12-18) months of age were brought from the local market of Basrah to be used. The rabbits were put in silk cages measuring (50 X 40X40) cm. in the animals house, in college of vet. Medicine of basrah university under temperature of (25$\pm$ 3) C$^{\circ}$ and relative humidity (50$\pm$5 %) .The animals are acclimatized for three weeks and maintained on regular feeding consisting of (crude protein 15%, ground soya bean 6%, wheat flours 50%, wheat bran 25%, saturated fat 2%, milk power 2% and minerals and vitamins *1g / Kg*). Water and food were given *ad labium.* The animals were fasted for 12 hrs. before blood collection and killing was carriedout . Male rabbits were randomly and equally divided into three groups (10 animals in each group)as following :

Group (A): The rabbits of this group recieved, oral administration (0.2 mg /Kg. body weight) from DEX daily for 60 days.

Group (B): The rabbits of this group recieved oral administration (1mg / kg. body weight) from DEX daily for 60 days.

Group (C): This group is the control group, rabbits of this group were given the distal water only as oral administration.

After (60) days from treatment, the animals were anestheticed by chloroform after dissection of neck, (adrenal gland, kidney and liver organ) were removed then these organs were fixed in 10%.formalin. The specimens were dehydrated through the increasing concentration of ethanol (50, 70, 80, 90, 90, 100 and 100%), followed by clearing with Xylene, infiltration of specimens and embedded in paraffin wax (blocking), (5-6) µm thick section were cut by using rotary microtome. All sections were put in water bath, (52 C^0) to plain the tissues then fixed on glass slides by Albumin Mayer (as a thin film) and placed for drying on the hot plate (40 C^0) for 24 hrs.Then staind by Harris Hematoxilin & Eosin (Luna, 1968).

Results

Adrenal Gland

The adrenal gland of the control group, appeared normal in their structure . This gland's surrounded by capsule and divided in two layers, the cortex and medulla. The cortex layer consist of three zones: zona glomerulosa, zona fasciculate, zona reticularis Figure (1).Meanwhile the adrenal gland of DEX treated rabbits revealed histopathological changes which included:

In group (**A**) the presence of pyknosis, degeneration, necrosis, and bleeding in the cells of zona glomerulosa and zona fasciculata, were observed (Fig.2 and 3).Group (**B**) showed necrosis, bleeding, Odem and pyknosis in zona glomerulosa and zona fasciculata and some effect in zona reticularis and medulla of gland (fig. 4 and 5).

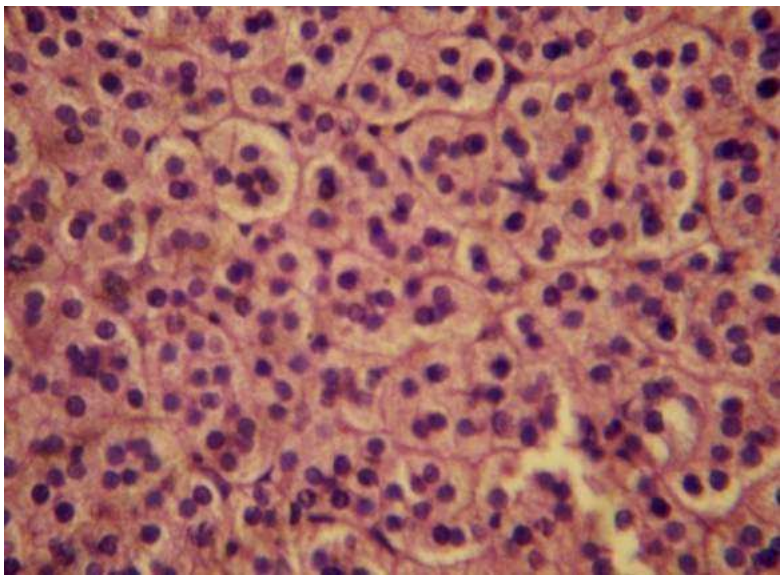

Figure 1: Transvere section through the adrenal gland of control group shows normal histological structure (H and E)(1200X)

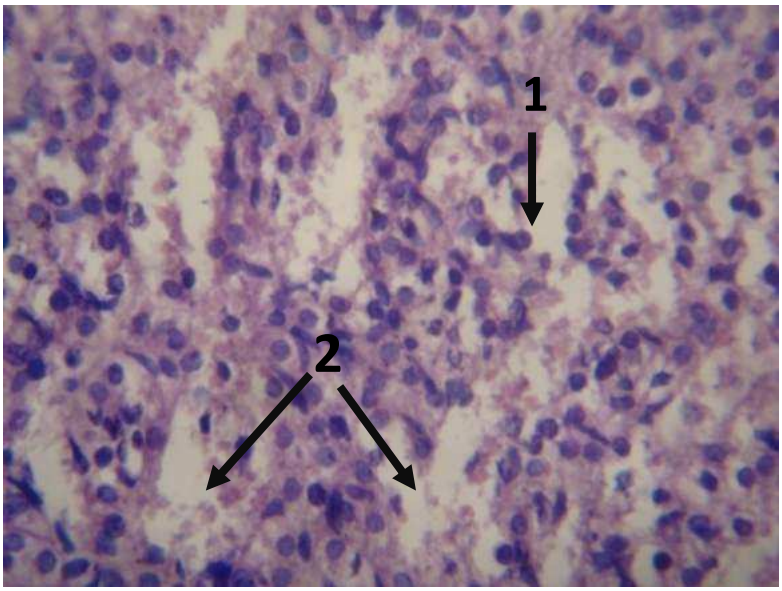

Figure 2: Transvere section through adrenal gland of 0.2mg/Kg DEX treated group. shows:1-pyknosis, 2-necrosis in the cells (H and E)(1400X)

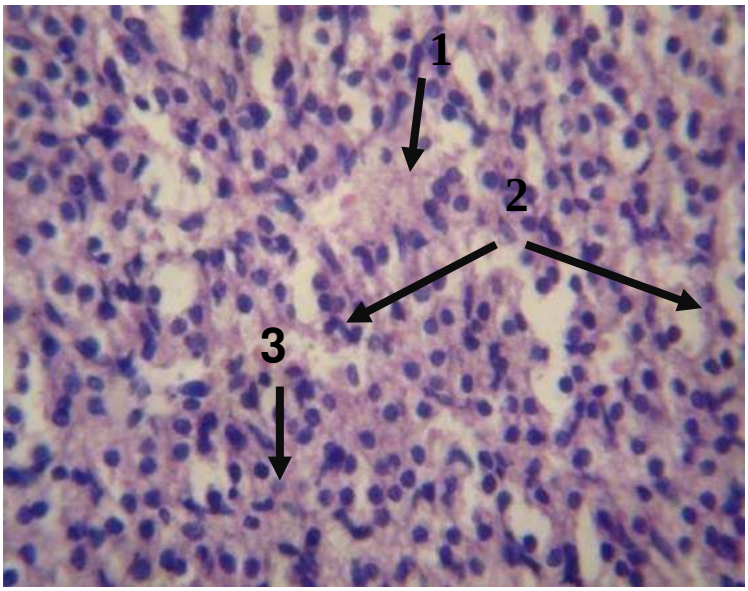

Figure 3: Transvere section through the adrenal gland of 0.2 mg/Kg DEX treated group shows: 1-odema, 2-necrosis 3-pyknosis, (H and E) (1400X)

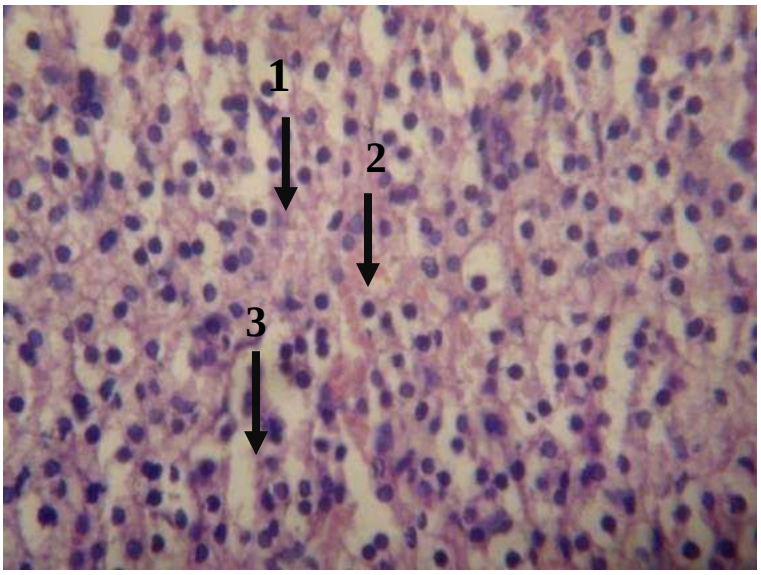

Figure 4: Transvere section through the adrenal gland of 1mg/Kg DEX treated groups . shows: 1-degeneration, 2-congestion 3-sinuses extension. (H and E) (1400 X)

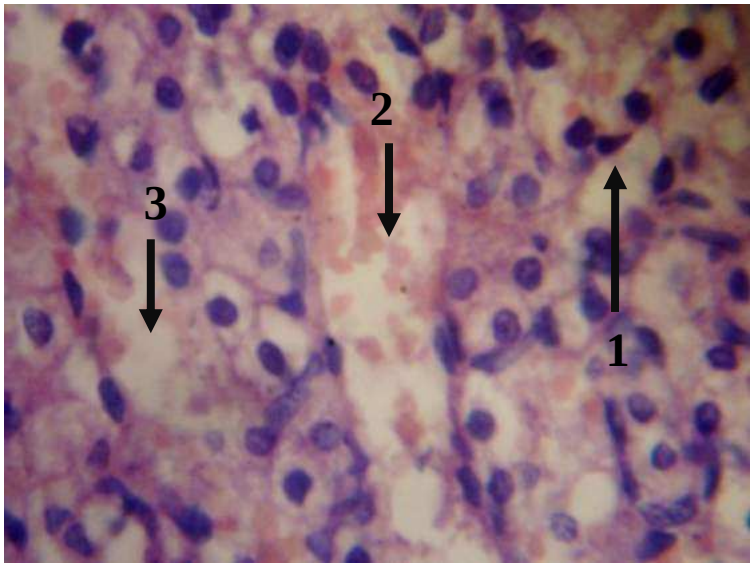

Figure 5: Transvere section through the adrenal gland of 1mg/Kg DEX treated groups . shows: 1-degeneration, 2-congestion 3-sinuses extension. (H and E) (1800 X)

Kidney

As shown in Fig. (6) kidneys of control rabbits show normal glomeruli, normal renal cortical tubules and normal epithelial cells lining of the renal tubules. While in the DEX treated animals the histological study of the kidney in the group (**A**) show fibrosis, necrosis, Odem and bleeding in the epithelial lining of convoluted tubules (Fig.7).Moreover atrophy, absence of glomeruli infiltration and accumulation of inflammatory cells were also found as shown in Fig. (8).Section of group (**B**) showed also odema, fibrosis, bleeding and infiltration of inflammatory cells in the convoluted and collecting tubules (Fig. 9). In addition to these damage the shrinkage and absence the glomeruli were recorded (Fig. 10).

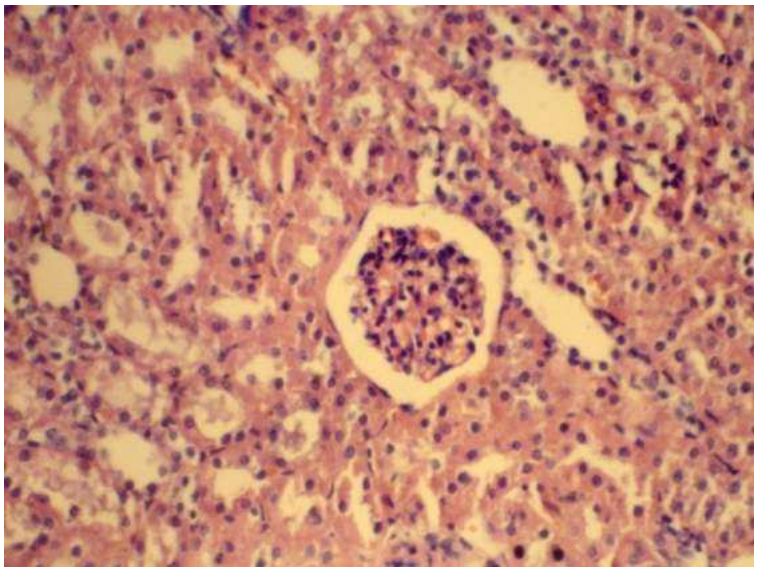

Figure 6: Transvere section though kidney of control group shows normal histological structure (H and E)(800X)

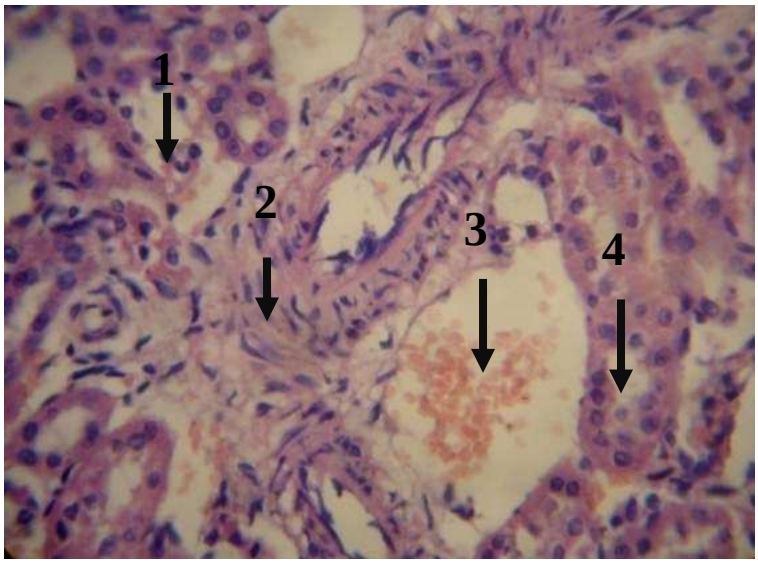

Figure 7: Transvere section through the kidney of group (A) shows : 1-necrosis of epithelial cells in convoluted tubules, 2-fibrosis, 3-congestion, 4-hyperplasia, (H and E) (1600 X)

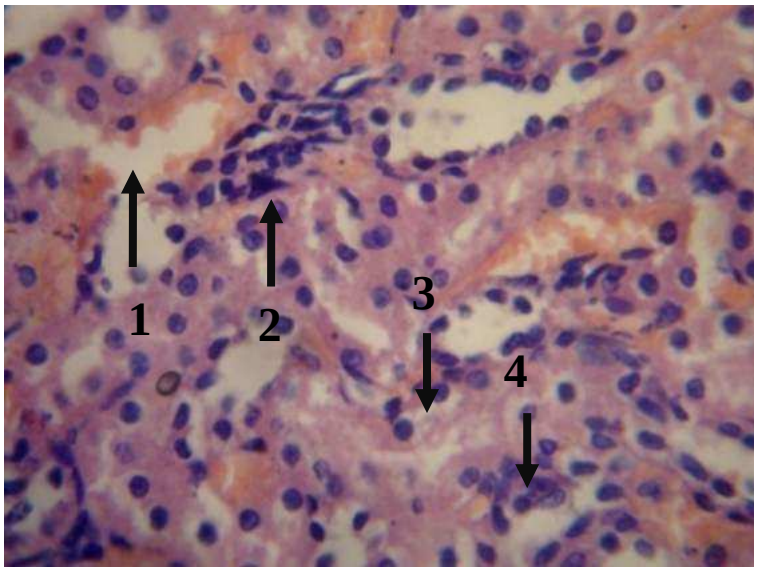

Figure 8: Transvere section though the kidney of group (A) shows :1-congestion 2-inflammatory cells aggregation , 3-odema, 4-hypertrophy, (H andE)(1600x)

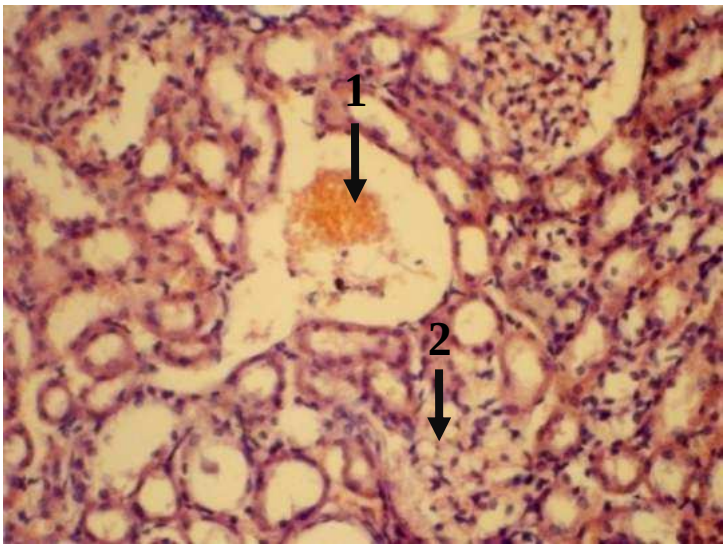

Figure 9: Transvere section through the kidney of rabbits treated with 1mg/Kg DEX showing :1-congestion , 2-odema, (HandE) (800 X).

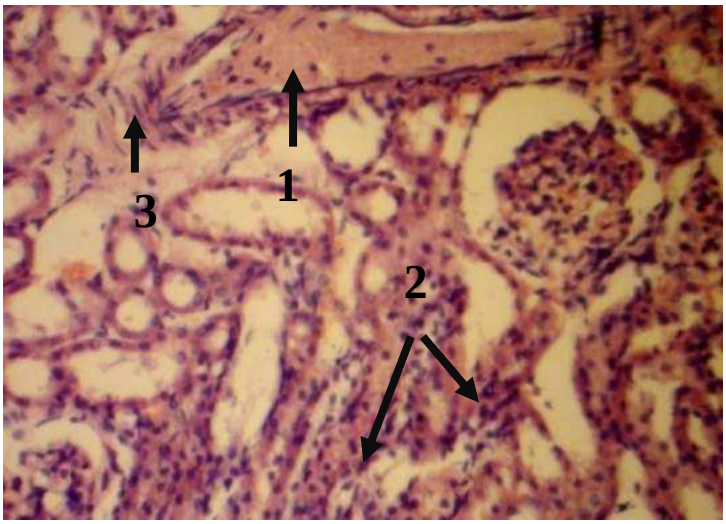

Figure 10: transvere section through the kidney of rabbits treated with 1mg/Kg DEX, showing :1-odema, 2-inflammatory cells aggregation , 3-fibrosis,(HandE)(800 X)

Liver

The liver of control group appeared normal structure of liver, which divided into the classical hepatic lobules. Each lobule was formed of cords of hepatocytes radiating from the central vein to the periphery of the lobule. The hepatic cells plates were separated by narrow blood sinusoids and normal hepatocytes (parenchymal cells) normal central vein with no congestion,and normal hepatic artery were observed. In addition, there is a cross section of normal branch bilary duct in fig. (11). However in the treated rabbits of group (**A**)the main histopathological changes which recorded are vacculation of hepatocytes, congestion, bleeding and extension in the sinusoids (Fig.12). Group (**B**) also show a congestion, bleeding, extension in the sinusoids and infiltration of inflammatory cells (Fig.13).

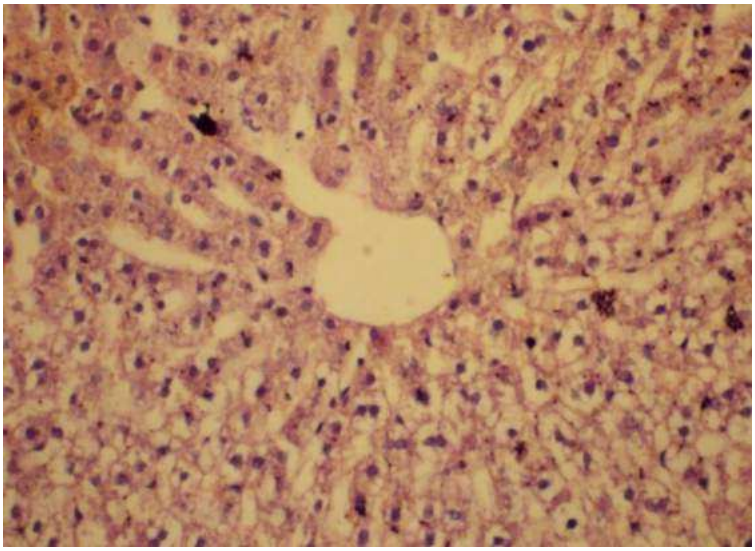

Figure 11: Transvere section through the liver of control group show normal histological structure, (HandE)(800x).

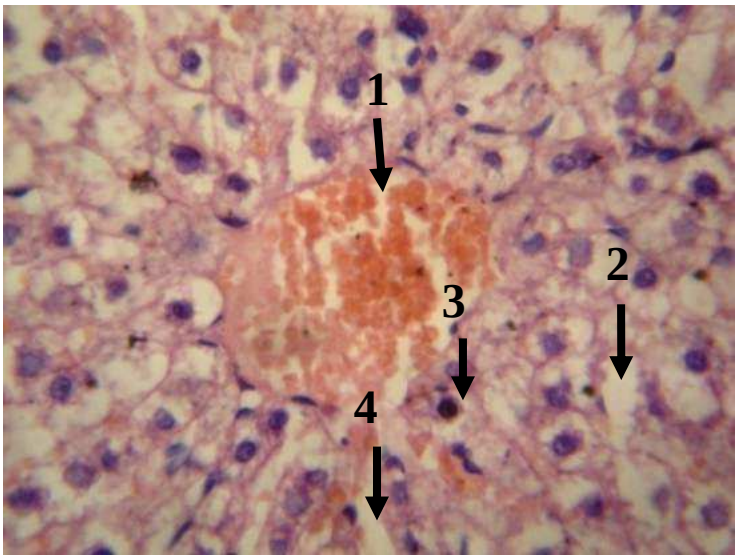

Figure 12: Transvere section through the liver rabbits treated with0.2mg/Kg DEX showing 1-congestion and bleeding, 2- vacillation, 3-neclues pyknosis, 4-sinuses extension,(HandE)(1400x)

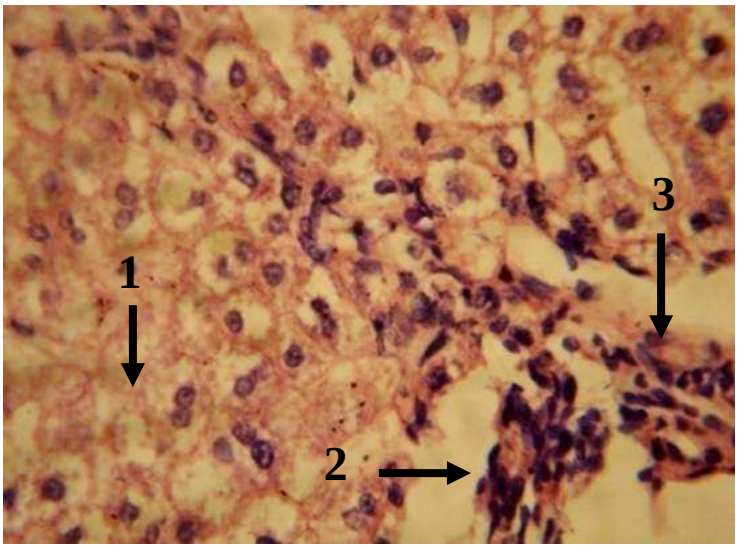

Figure 13: Transvere section through the liver rabbits treated with1mg/Kg B.W DEX showing 1-degeneration, 2-fibrosis, 3-inflammatory cells aggregation , (HandE)(1400X).

Discussion

Effect of DEX. on Adrenal Gland Structure

This study shows that treatment of male rabbit with DEX resulted in histopathological changes in the structure of adrenal gland which may be due to the effect of DEX on the adrenal gland function. Messer *et al*., (1983) observed that the administration of DEX in a large dose is associated with depression of many aspects of the adrenal function.The histopathological changes including reduction of absolute and relative adrenal mass due to reduction of cortical mass specially that of Zona Fasciculata. This was expected because glucocorticoid synthesis is performed most in the zona fasciculata that it is the largest part of the cortex(Margarita *et.,al* 2006).Another lesion note in the present study is pyknosis of nucleuse in some cells, degeneration and fibrosis in the zona fasciculata and reticularis. All these changes occures due to loss of regular structuring of the cells and cell columans as a result for adrenal atrophy occur after DEX treatment,(Mughal *et al.,* 2004). It is well known that adrenocorticotropic hormone(ACTH) affects growth and endocrine function of the adrenal cortex and zona fasciculata cells secret glucocorticoids under the regulation of ACTH (Mitani *et. al*., 2003). The

release of ACTH is influenced by a wide variety of internal and external factors such as stressors and glucocorticoids (Wang *et. al.,* 2008). Dexamethasone suppresses the hypothalamic-pituitary-adrenal (HPA) axis therfore (Cunha *et al.,* 2004) reported that DEX treatment result in rapid adrenal gland atrophy. This phenomenon could be explained by the induction of programmed cell death. Glucocorticoids (especially DEX) are known to induce apoptosis and reduce proliferation in a variety of different cells (Ranta *et. al*., 2006). The result of the present study not showed histological changes in the medulla of gland which is innervated by presynaptic sympathetic nerve fibers and release catecholamine . The release of these "adrenergic or sympathetic" transmitters into the blood stream amplified the effect of the sympathetic branches of the outonomic nervous system at virtually all of the target organs of the sympathetic nervous system .(Guyton and Hall, 2004).

Effect of DEX. on Kidney Structure

Histopathological changes of the rabbit kidney tissue treated with DEX in dose (1mg./kg. B.W) was prominent and characterized by increase the size and number of perirenal adipocytes.This result was in agreement with (Latif, 2010) in his study on rat he explained that due to DEX effect on lipids metabolism. Another changes include fat necrosis, bleeding, fibrosis in the epithelial lining of the convoluted tubules, these effects were observed because the long time of DEX administration (60 days) as a chronic septum accompained with increases insulin resistance increase gluconeogensis and peripheral glucose utilization may be decrease so that hyperglycaemia and sometimes glucoseuria might be resulted.(Bennett and Brown, 2003). Glomeruli atrophy and glomeruli absence sometimes appeared in section obtained from rabbit kidney in group (B).

Effect of DEX. on Liver Structure

Histopathological changes of the liver treated with DEX showed a many histopathological changes in hepatocytes and sinusoids, These change represented by disruption of hepatic cords, degeneration and necrosis of hepatocytes, appearance of inflammatory cells and congestion sinusoids and ballooning found in the degenerative hepatocytes, concentric periductular fibrosis around bile ducts, leading eventually to destruction of affected bile ducts and obstruction of other. Many fields of liver histological sections revealed cirrhotic and fatty changes**.**

These results were in agreement with (Kim and shin,1998), who note the ballooning of hepatocytes at mid-and periportal zones in dog liver treated with dexamethasone and explain that due to the accumulation of intracellular fluids which caused hydropic swelling.

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دراسة التغيرات النسيجية الامراضية في بعض الأعضاء الناجمة عن العلاج طويل الأمد بالدكساميثازون في ذكور الأرانب المحلية.

الخلاصة

الهدف من إجراء الدراسة هو تحديد التغيرات النسيجية الامراضية في الغدة الكظرية, الكلية والكبد والناجمة عن المعالجة بالدكساميثازون لفترة طويلة في ثلاثين أرنبا ذكرا محليا في مدينة البصرة قسمت عشوائيا إلى ثلاثة مجاميع, جرعت مجموعة السيطرة (ج) بالماء المقطر فقط طيلة فترة التجربة. جرعت المجموعة الأولى (ا) بجرعة (٠,٢ ملغم./ كغم. وزن الجسم) يوميا لمدة (٦٠) يوما بينما جرعت المجموعة الثانية (ب) بجرعة (١ ملغم./كغم. وزن الجسم) يوميا لنفس الفترة (٦٠) يوم. اظهر الفحص النسيجي باستخدام المجهر الضوئي وجود العديد من التغيرات النسيجية المرضية في الغدة الكظرية للأرانب في مجموعتي المعاملة تمثلت بوجود الاحتقان في الأوعية الدموية وتنكس, تفجي وتنخر في خلايا النطاق الكبيبي والنطاق الحزيمي لقشرة الغدة. أظهرت الدراسة أيضا وجود العديد من التغيرات في كلية الارانب في مجاميع المعاملة تمثلت بوجود التنكس, الوذمة والتنخر في خلايا البطانة الطلائية للنبيبات الكلوية كما لوحظ ارتشاح الخلايا الالتهابية وضمور في بعض الكبيبات الكلوية. تمثلت التغييرات النسيجية المرضية في الكبد بانتفاخ سايتوبلازم بعض الخلايا الكبدية واتساع الجيبانيات.

LIPID PROFILE AND HEMATOLOGICAL CHANGES IN GALLSTONE PATIENTS

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Abstract

The current study was aimed to find out the possible alterations of the serum lipid profile and some hematological parameters in 17 (11 female and 6 male) gall stone former patients with age range between (19-57) years. During this study, total serum cholesterol, triglycerides, lipoproteins (HDL and LDL) and total leukocyte count, erythrocyte count, hemoglobin ratio and platelet count were included. All cases were newly diagnosed in surgery unit of Rizgary hospital in Erbil city. The results showed an elevation in the level of cholesterol, triglyceride and LDL and decreased cholesterol-HDL level in patients with cholelithiasis. Furthermore, the total erythrocyte number, total leukocyte number and platelet count were increased with a significant decrease of blood haemoglobin levels in the gallstone patients. In conclusion the changes in the levels of lipid profile and hematological parameters may be used as markers for gallstone formation.

Key word: *Gallstone, Lipid profile, Hematological parameters*

Introduction

Gallstones or cholelithiasis is the most common type of gallbladder diseases affecting 10% of USA population (Dave, 2010). The main constituents of gallstone are cholesterol, bilirubin and calcium (Johnston and Kaplan, 1993). Other constituents may include fatty acids, triglycerides, protein and polysaccharide (Selvaraju *et al*., 2009).

Gallstones are crystalline structures formed by concretion or accretion (adherence of particles,accumulation) of normal or abnormal bile constituents through three stages of gallstone formation, supersaturation, nucleation and aggregation (Channa, 2008).

It is now widely accepted that the primary event in the pathogenesis of cholesterol gallstones is an altered lipid metabolism because of which there is a relative increase in the cholesterol levels are compared to other lipids secreted by the liver into the bile (Rao *et al*., 2012). Alterations in the lipid metabolism may arise as a result of a combination of various factors such as estrogen treatment, aging (Cuevas *et al*., 2004), hypertriglyceridemia, over weight and insulin resistant patients (Smelt, 2010), excess dietary cholesterol/fat, obesity, diabetes and genetic causes (Rao *et al*., 2012). Women are twice as likely as men to develop gallstones; the higher prevalence of gallstones in women is thought to be caused by multiple pregnancies and obesity (Devrajani *et al*., 2010).

Lithogenicity of the bile is mainly determined by the concentrations of their principal three lipid components, cholesterol, bile acids and phospholipids (Smelt, 2010). Relative increase concentrations of cholesterol in bile, or supersaturation, led to gallstones formation (Cuevas *et al*., 2004) also changes in bile acid metabolism and gallbladder function are critical factors in the pathogenesis of gallstones diseases (Smelt, 2010).

Some previous studies reported that advanced age, history of coronary artery disease, and leukocytosis have to be associated with an increased risk for gangrenous cholecystitis (Fagan *et al*., 2003).The gall stone may results in hemorrhagic cholecystitis with hemoperitoneum and depletion of Hb content, which is most familiar in female (40%) than in male (36%) (Channa *et al*., 2005). In another study (Kim *et al*., 2007) showed that gallstone spillage caused by the perforation of the hemorrhagic cholecystitis which cause the drop of blood hemoglobin also thrombocytopenia. It was also revealed that in patients with hemoglobin C diseases, continued hemolysis may produce pigment gallstones which are unusual type of gallstone (Carter and Besa, 2012).

In general, beside the determination of lipid profile for prognosis of patient with gallstones, a routine hematological and liver function tests in preoperative assessment of uncomplicated symptomatic cholelithiasis usually required (Habib *et al*., 2009). This is due to the fact that gallstone disease is known to cause liver disease and a derangement of its enzymes (Olokoba *et al*., 2009). This may due to move of gallstone into the common bile duct and liver, causing inflammation, and elevated leukocyte count and

liver enzymes often result from damage to liver cells caused by inflammation and infection (Pereira-Lima *et al.*, 2000).

This study was aimed to find out the possible change in serum lipid profile and some hematological parameters in gall stone formation.

Materials and Methods

During this study, total serum cholesterol, triglycerides, lipoproteins (HDL and LDL) and some hematological parameters (Total leukocyte count, erythrocyte count, Hb value and platelet count) were studied in the sera of 17 patients with gallstones newly diagnosed in surgery unit of Rizgary hospital- Erbil, the patients were of normal weight, non smokers, not receiving any treatments and with age range between 19-57 years.

Fasting blood samples were collected by drawing five mls of venous blood from each patient and healthy persons. The samples were immediately transferred into plastic tubes, centrifuged at 3000 rpm for 15 min and the serum was separated and kept at – 85O C until assay. The serum cholesterol level was estimated using standard kit (Syrbio, France), which depends on enzymatic conversion of cholesterol to quinoneimine pigment, then its absorbance was measured at a wave length of 500 nm using UV/VIS Spectrophotometer (Philips, Pye Unicam SP800). Serum triglyceride level was estimated using standard kit (Syrbio, France), which depends on enzymatic hydrolysis triglyceride with lipases, then its absorbance was determined at wave length of 520 nm using UV/VIS Spectrophotometer (Philips, Pye Unicam SP800). LDL- cholesterol and HDL-cholesterol were measured according to Sewerynek (2000).

Total leukocyte, erythrocyte and platelet counts were determined using hemocytometer. Hemoglobin (Hb) concentration determined using Drabkin solution by cynmethaemoglobin method.

Data was analyzed by using student T-test by statistical packages for social science software (SPSS). Statistical analysis with a value greater than 0.05 considered as non significant (NS) and less than 0.05 considered as significant.

Results

The results in (table 1) shows significant ($p<0.05$) elevation in total cholesterol, triglyceride and LDL levels in patients with cholelithiasis when compared to the healthy control persons. While the level of serum HDL was significantly ($p<0.05$) decreased in gallstone patients as compared with healthy individuals.

Table (1): Change in lipid profile in gallstone patients

Lipid profile	Control(No= 10)	Patients(n=17)
Cholesterol	144.40 ± 5.01	225.94 ± 6.97*
Triglycerides	86.60 ± 4.79	305.88 ± 10.51*
LDL	113.10 ± 4.78	173.17± 5.66*
HDL	43.10 ± 1.57	35.82± 1.84*

***P<0.05: significant**

The results in table (2) shows that there are significant ($p<0.05$) increases in the total erythrocyte (TEC), total leukocyte (TLC) and platelet counts in gallstone patients as compared to the healthy control persons. On the other hand there is a significant decrease in haemoglobin (Hb) level in gallstone patients than the healthy control group.

Table (2): Hematological parameters in control and gallstone patients

Hematological parameters	Control (No= 10)	Patients (n=17)
TLC* 10^3	7.0±0.2	7.5 ±0.3*
TEC* 10^6	5.0 ±0.1	5.7±0.3*
Hb (gm/dl)	14.4 ±0.4	13.4±0.2*
Platelets	210.9 ±6.2	275.4±12.2*

***P<0.05: significant**

Discussion

Increased total lipid profile (cholesterol, triglyceride and LDL) levels in gallstone patients agree with the results of gallstone patients in Pakistan reported by Channa *et al*., (2010), Channa (2008) showed that free cholesterol increases steroid synthesis, which inturn decreases the production of bile acids is responsible for the precipitation of cholesterol in bile, which is a prerequisite for gallstone formation. Jaraari *et al*., (2010) also revealed that quantitative analysis of Libyan patients serum with gallstone showed significant increase of cholesterol level. Shiina *et al*., (2011) showed that there was a significant increase in total free cholesterol, cholesterol-LDL, and triglycerides in patients with cardiac disease developed gallstones after cardiac surgery. Some investigators reported a positive association between gallstone and serum triglycerides in Spanish men (Channa *et al*., 2010) whereas; others found no such association (Olokoba *et al*., 2006). In Libyan patients with cholelithiasis unlike that of other studies both male and females serum showed highly significant raise in total cholesterol, LDL and HDL level (Rao, *et al*., 2012), But our results showed decreased level of cholesterol-HDL in studied patients group.

The significant increase in total leukocyte in gallstone patients may due to the fact that liver produces bile and releases it to the gallbladder through the common bile duct, gallstones can eventually move into the common bile duct and liver, causing inflammation, and elevated leukocyte count and liver enzymes often result from damage to liver cells caused by inflammation and infection (Pereira-Lima *et al*., 2000). This is consistent with the results of Merriam *et al*., (1999); Barak *et al*., (2009) and (Black and Hawks, 2004) found that the elevated leukocytosis appears to be related to the intense inflammatory reaction associated with the gangrenous cholecystitis which inturn caused elevated total WBC level (Black and Hawks, 2004). Channa *et al*., (2005) showed that the depletion of blood hemoglobin is most familiar in gall stone patients, while the blood TLC rose in gallstone patients. This agree with the result of the present study, in which there is significant increase in the total platelet and erythrocyte count, and decrease in Hb in studied gall stone patients. While Kim *et al*., 2007 showed that blood analysis of newly diagnosed gallstone patient revealed mild thrombocytopenia and decrease in Hb-value and increase WBC–count this was because gallstone spillage caused by the perforation of the hemorrhagic cholecystitis which cause inflammation and increasing WBC in blood and drop of blood hemoglobin also thrombocytopenia.

Increase in total platelet count in the present study is a good indicator of presenting hemorrhage, because platelets play an important role in spontaneous homeostasis, interrupting in a natural manner the hemorrhage resulting from the traumatic rupture of blood vessels and these cells act on the acute phase of the inflammatory response that occurs in response to tissue injury, releasing vasoactive amines (histamine and serotonin) that are chemical mediators of inflammation (Crema *et al*., 2005).

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پوختە:

لەم لێکۆڵینەوەی ئێستادا پشکنین بۆ گۆڕانکاریەکانی چەوری خوێن لە جۆرەکانی (Triglyceride Total ,cholesterol, HDL, LDL) و ڕێژەی پێکهاتەکانی خوێن لە (خڕۆکەی سپی, خڕۆکەی سوور, پەڕەکانی خوێن, ڕێژەی هیمۆگلۆبین) ئەنجام درا لە کەسانی تووش بوو بە بەردی زراو. ئەم پشکنینە ئەنجام درا بە وەرگرتنی سیرۆمی ١٠ کەسی ساخ و ١٧ نەخۆشی تووشبوو بە بەردی زراو (١١ مێ) و (٦ نێر) دوای سەلماندنی نەخۆشیەکە لە یەکەی نەشتەرگەری گشتی نەخۆشخانەی رزگاری فێرکاری لە شاری هەولێر .

هەڵسەنگاندنی ئەنجامەکانی لێکۆلینەوەکە لە کەسانی نەخۆش بە بەراوردکردن لەگەڵ کەسانی ساخ (کۆنترۆڵ) بەرزبوونەوەیەکی بەرچاو دەر دەخات لە چەوریەکانی جۆری (Total cholesterol, Triglyceride and LDL,) ی سیرۆمی نەخۆشەکان,بەڵام ئاستی چەوری جۆری (HDL) لە هەمان ئەو نەخۆشانەدا نزمە , لەگەڵ ئەوەشدا زیابوونێکی بەرچاو لە ڕێژەی (خڕۆکەی سپی, خڕۆکەی سوور, پەرەکانی خوێن) بینرا لەگەڵ نزم بوونەوەی ئاستی هیمۆگلۆبینی خوێنی هەمان ئەو نەخۆشانەدا.

الخلاصة

في هذه الدراسة، تم اختبار ألتغيرات المحتملة للدهون في الدم وبعض مكونات الدم في تكوين الحصوه. اذ تم دراسة مستوى الكولسترول ، الدهون الثلاثية، البروتينات الدهنية (HDLوLDL) والعدد الكلي لخلايا البيض وعدد كرات الدم الحمراء والمحتوى الهيموغلوبيني للدم وعدد الصفائح الدموية, و شملت الدراسة ١٧ مريضا (١١ أنثى و ٦ ذكور) باعمار بين (١٩–٥٧) سنة. تم تشخيص جميع الحالات المرضى حصاة المرارة في وحدة جراحة في مستشفى رزطارى التعليمى في مدينة اربيل.

أظهرت النتائج ارتفاعا في مستوى إجمالي للدهون (الكولسترول، والدهون الثلاثية و LDL) و انخفاض مستوى الكولسترول HDL–في المرضى الذين يعانون من تحص صفراوي. كما أظهرت النتائج زيادة في كرات الدم الحمراء , عدد الصفائح الدموية وخلايا الدم البيضاء وانخفاض ملحوظ في مستويات الهيموكلوبين في الدم المرضى اللذين يعانون من تحص صفراوي.

TOXIC EFFECTS OF MILLER ON CHROMOSOMES AND SPERMS OF ALBINO MALES MICE

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Abstract

The genotoxicity of Miller (glyphosate) herbicide was evaluated in males albino mice *Mus muscles* BALB/c strain. The tested parameters were chromosomal aberrations and sperm abnormalities to evaluate a possible damage effects on genetic material and sperms. The herbicide was administered orally to adult male laboratory mice in 4 different doses (0, 250, 500, and 750 mg/kg of body weight). The mice were killed at two periods: after 3 and 6 weeks. The results of chromosome aberration assay revealed that all the tested doses and periods induced structural chromosomal aberrations (CAs) such as centromeric gaps, chromatid gaps, delations, dicentric chromosomes and ring chromosomes. The results of sperm abnormality assay revealed that miller has the ability to induce sperm abnormalities in all doses used comparing to untreated mice, which represented by hookless sperm, swollen head sperm, amorphous head sperm, sperm without head, defective hook sperm, banana head sperm, double head sperm, sperm without tail, double tail sperm, bent midpiece defect and coiled tails.

Key words: glyphosate, chromosome aberrations, sperm abnormalities, herbicide.

Introduction

Pesticides, including herbicides, insecticides, and fungicides are used extensively to improve crop yields and as a result, they accumulate in the environment and human unavoidably exposed to them (Van der Werf, 1996). Studies show that toxic residues may rise about ten times through the food chain due to biological magnification in the body fat of living organisms and becoming more concentrated as they move from one creature to another, because living organism cannot get rid of these toxins by their biological metabolism (Cornell university: pesticide fact sheets, 2007). Because of their biological activity, the indiscriminate use of pesticides may cause undesired effects to human health, for instance, the induction of DNA damage can potentially lead to adverse reproductive outcomes, or induction of cancer, and many other chronic diseases (Lander *et al.,* 2000 and Zhao *et al.*, 2002). Most of pesticide had been tested for their genotoxicity and carcinogenicity using different testing assays.

The United States Environmental Protection Agency (U.S EPA 1993) classified glyphosate as Group E, evidence of non carcinogenicity in humans, based on studies of laboratory animals that did not produce compelling evidence of carcinogenicity. Most investigations in lab animals presented in glyphosate Technical Fact Sheet published by National Pesticide Information Center (Miller *et al*, 2010) confirmed non-carcinogenicity effect of glyphosate in the low-doses and mid-doses, but in high-doses, slightly increased incidence of renal tubular adenomas, increased incidence of hepatocellular hypertrophy, hepatocellular necrosis and interstitial nephritis. However the EPA and an independent group of pathologists and biometricians concluded that the occurrence of adenomas was not caused by glyphosate, as well glyphosate was negative in various *in vivo* and *in vitro* test systems evaluating gene mutation, chromosomal aberration and DNA damage (Cal/EPA, 1992).

Miller or glyphosate [N-(phosphonomethyl) glycine - isopropylamine (IPA) salt; $C_3H_8NO_5P$] is a post-emergent, non-selective phosphonoglycine herbicide. In 1970 it was discovered to be a herbicide by the chemist John E. Franz of Monsanto company,USA. In 2001 considered the most heavily used herbicide on crops, in forestry and in residential settings. In plants, glyphosate disrupts the shikimic acid pathway through inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate(EPSP) synthase. The resulting deficiency in EPSP production leads to reductions in aromatic amino acids that are vital for protein synthesis and plant growth (Vencill, 2002). The shikimic acid pathway is specific to plants and some microorganisms. The absence of this pathway in mammals may explain the low toxicity of glyphosate to non-target organisms(WHO 1996). Glyphosate is not absorbed by a plant's root system because of its strong adsorption to the

soil. However, it is easily absorbed by leaves from spray residues and translocated throughout the plants and fruits. Therefore, glyphosate concentration may increase in plants immediately after spray. Lettuce, carrots, and barley contained glyphosate residues up to one year after the soil was treated with 3.71 pounds of glyphosate per acre (McMullan, Honeggar, and Logusch, 1990). In soils, glyphosate is readily degraded by soil microbes to inorganic constituents, including carbon dioxide and phosphate. However, no appreciable degradation of glyphosate was observed in water via chemical, microbiological or photolytic processes for 78 days (Anton *et al.*, 1993). Chromosome aberrations, sister chromatid exchange (SCE), and mitotic index were studied in peripheral lymphocytes from three healthy donors exposed *in vitro* to different concentrations of glyphosate. Glyphosate produced a dose-related increase in the percent of aberrant cells and an increase of SCE per cell, but did not affect the mitotic index (Lioi,*et al.* 1998). In another research, Prasad *et al.,*(2009) observed that Glyphosate treatment significantly increased chromosomal aberrations (CAs) and micronuclei (MN) in bone marrow cells of Swiss albino mice. Poletta *et al*. (2009) evaluate the genotoxic potential of glyphosate in erythrocytes of broad-snouted Caiman (*Caiman latirostris*) using Comet assay and MN test. A significant increase in DNA damage was observed at a concentration of 500 µg/egg or higher, compared to untreated control animals. Results from both the Comet assay and MN test revealed a concentration-dependent effect.

Effects of Pesticides on Sperm

The survival of a species depends on the integrity of its reproductive system. Damage by physical or chemical agents to the sperm, ovum or fertilized ovum may cause infertility, spontaneous abortion and birth defects, or may result in mutations that are passed on to future generations (Swati, 2004). Chlorpyrifos at dose levels of 7.5, 12.5 and 17.5 mg/kg BWT/day was administered orally to male rats for 30 days to evaluate the toxic alterations in testicular histology, biochemistry, sperm dynamics and testosterone levels, the results concluded that Chlorpyrifos induces severe testicular damage and results in reduction in sperm count and thus affect fertility (Joshi *et al*., 2007). Clair *et al.* (2012) found that glyphosate at higher doses provoke necrosis and apoptosis in germ cells and membrane degradation in Sertoli /germ cells as well decrease testosterone by 35% at lower doses.

In the literature there are a lot of other examples showing the adverse effects of different types of pesticides on the genetic materials and sperms of different mammals. In contrast to these data Bayer Glyphosate 450 Herbicide Product safety sheet data (2010) indicate that this herbicide is practically non-toxic to fish, aquatic invertebrates, birds and bees and has low toxicity to earthworms. Dietary concentrations of up to 10,000 ppm or 293 mg/kg/day of glyphosate given to rats over two generations had no effect on male or female sexuality and fertility. The lowest observed adverse effect level (LOAEL) for parental and offspring toxicity is 3000 ppm, based upon a reduction of body weight at 10,000 ppm (Moxon, 2004). Spermiotoxicity study by Akcha *et al.* (2012) revealed that glyphosate had no cytotoxic effects on oyster spermatozoa.

These data presents inconsistent and conflicting results on the effects of glyphosate. A report of Buffin and Jewell (2001) on health and environmental impacts of glyphosate as well had included a lot of divergence and controversial data. The aim of this research is to investigate the effects of glyphosate (Miller) on chromosomes and sperms of mice to clarify some of these conflicts.

Material & Methods

Reagents

Miller, in the form of Glyphosate 480 g / Liter was brought from local markets of Dohuk city. The acute oral LD_{50} of Miller to male albino mice was found to be 1568 (mg/kg bw) (NTP, 1992). Miller was used in three doses (250, 500, and 750) mg/kg of body weight. The doses were prepared by diluting the herbicide with distilled water then placed in clean and dry bottles. The mice were treated orally 3 times weekly by using dosage syringe prepared locally from 2 ml disposable syringe and needle.

Animals

Adult males of Swiss albino mice (*Mus musculus*) BALB/c (8-10) weeks in age, weighing (30-35) gm were used in this study. All aspects of the animal experiment, breeding, parturitions were carried out in the Animal House of the Department of Biology, Faculty of science, University of Zakho and maintained at

room temperature (22 ± 2).A standard diet and water was used to feed the mice.

Preparation of chromosomes from the bone marrow cells

At the end of the treatment, each animal was injected intraperitoneally with 1ml of fresh colchicine (0.04 %) to arrest cell division at metaphase. Two hours after injection, animals were sacrificed by cervical dislocation for preparation of the chromosomes from bone marrow cells. Chromosomes were prepared by using the methodology of Evans *et al*. (1964).The slides were air dried at room temperature, stained with 2 % Giemsa stain for 10-15 min then washed with phosphate buffer to remove the excess stain. At least 100 metaphase cells per animal were scored to investigate chromosomal aberrations (Sharma and Sharma,1980).

Sperm preparation

The sperms were prepared from epididymis and vas deferens. After killing the animals, the epididymis and vas deferens were removed from the reproductive system and transferred to a small Petri dish containing normal saline. Using a sharp scissor the epididymis and vas deferens were cut into several parts, and the sperms were released into saline solution. The sperm suspension was smeared, dried, fixed with fixative (three volumes of absolute methanol and one volume of glacial acetic acid.), then stained with haematoxylin for 15 min, washed with tap water, then stained with 1 % eosin for 10 min and washed with tap water and left to dry at room temperature. At least 1000 sperms were counted from each animal to determine sperm morphology and abnormalities (Wyrobek, 1979).

Experimental design and Statistical analysis

The experiment was carried out in a factorial design arranged in a Completely Randomized Design (CRD) .The main factors were: **A-** Miller doses, the concentrations were (0.0, 250, 500, and 750 mg/kg b.w.). Each treatment was repeated 5 times. **B-** Periods (P_1 =3weeks, P_2=6 weeks).

Statistical analyses were performed with SAS software. Data were analyzed using one way analysis of variance (ANOVA) followed by Duncan's multiple range of the doses and periods (Duncan, 1955). Results were reported as mean values ± S.E. and differences were considered as significant at ($P \leq 0.05$).

Results

Effects of Miller on chromosomes of bone marrow cells of mice

The frequencies of different types of structural chromosomal aberrations (CA) induced by the herbicide miller in bone marrow cells of mice are shown in Table (1). The results show a significant differences ($p<0.01$) in chromosomal aberrations such as centromeric gaps, chromatid deletion, chromatid gaps, dicentric chromosome and ring chromosome when compared to untreated mice. These types of aberrations are shown in Figure 1. The data in Table 1 also reveal great differences in the total percentage of aberrations. These values increased with increase of miller doses as well with extending of the treatments period. The results in Table 1 indicate that the most types of aberrations were the centromeric gaps and ring chromosomes at D3 5.60±0.47 and 7.20±0.71 respectively when compared to control D0 2.50±0.42 and2.10±0.23, respectively. The least CA was the dicentric chromosomes in control group 0.30±0.15 increased to 0.60±0.16 in D3.

The number of CA increased with extending of the doses period as shown in table (1). There was a highly significant effects ($p<0.01$) of periods on centromeric gaps, chromatid gaps and ring chromosomes and significant effects ($p<0.05$) on chromatid deletion, while the periods didn't shows any effects on the formation of dicentric chromosomes.

Significant interactions ($p<0.05$) between doses and periods has been found in ring chromosomes while interaction in the rest of the parameters were non significant (Table 1).The highest interaction value scored in D3P2 8.80±0.86 for the ring chromosome. The data in table (1) as well shows that the total values of interactions increased with the increase of miller doses as well with extending of the treatments period.

Table (1): Mean values of different chromosomal aberrations induced by miller in bone marrow cells of Swiss male mice

	Factors	Chromosome Aberrations					
		Centromeric gap	Chromatid delation	Chromatid gaps	Dicentric chromosome	Ring chromosome	Total-percent aberrations
Doses	D0(control)	2.50±0.42b	0.20±0.13b	1.10±0.10b	0.30±0.15	2.10±0.23c	4.1
	D1	4.60±0.37a	1.30±0.26a	3.40±0.37a	0.20±0.13	4.70±0.39b	9.5
	D2	4.70±0.44a	1.50±0.22a	3.80±0.41a	0.40±0.16	5.40± 0.30b	10.4
	D3	5.60±0.47a	2.00±0.29a	4.00±0.42a	0.60±0.16	7.20±0.71a	12.2
	Significant	p<0.01	p<0.01	p<0.01	N.S	p<0.01	
Periods	P1	3.90±0.35b	1.00±0.17b	2.55±0.27b	0.35±0.10	4.10±0.36b	11.9
	P2	4.80±0.40a	1.50±0.24a	3.60±0.39a	0.40±0.11	5.60±0.59a	15.9
	Significant	p<0.05	p<0.05	p<0.01	N.S	p<0.01	
Interactions(Doses and Periods)	D0P1	2.40±0.60	0.20±0.20	1.00±0.00	0.40±0.24	2.00±0.31d	6
	D0P2	2.60±0.67	0.20±0.20	1.20±0.20	0.20±0.20	2.20±0.37d	6.4
	D1P1	4.20±0.58	1.00±0.31	2.80±0.37	0.20±0.20	4.00± 0.44c	12.2
	D1P2	5.00±0.44	1.60±0.40	4.00±0.54	0.20±0.20	5.40±0.50bc	16.2
	D2P1	4.20±0.66	1.20±0.20	3.20±0.58	0.40±0.24	4.80±0.37bc	13.8
	D2P2	5.20±0.58	1.80±0.37	4.40±0.50	0.40±0.24	6.00±0.31b	17.8
	D3P1	4.80±0.66	1.60±0.40	3.20±0.37	0.40±0.24	5.60±0.50b	15.6
	D3P2	6.40±0.50	2.40±0.40	4.80±0.58	0.80±0.20	8.80±0.86a	23.2
	Significant	N.S	N.S	N.S	N.S	p<0.05	

Note : Similar letters in each column refer to non significant difference while different letters refer to significant difference between them.
N.S : Non significant.

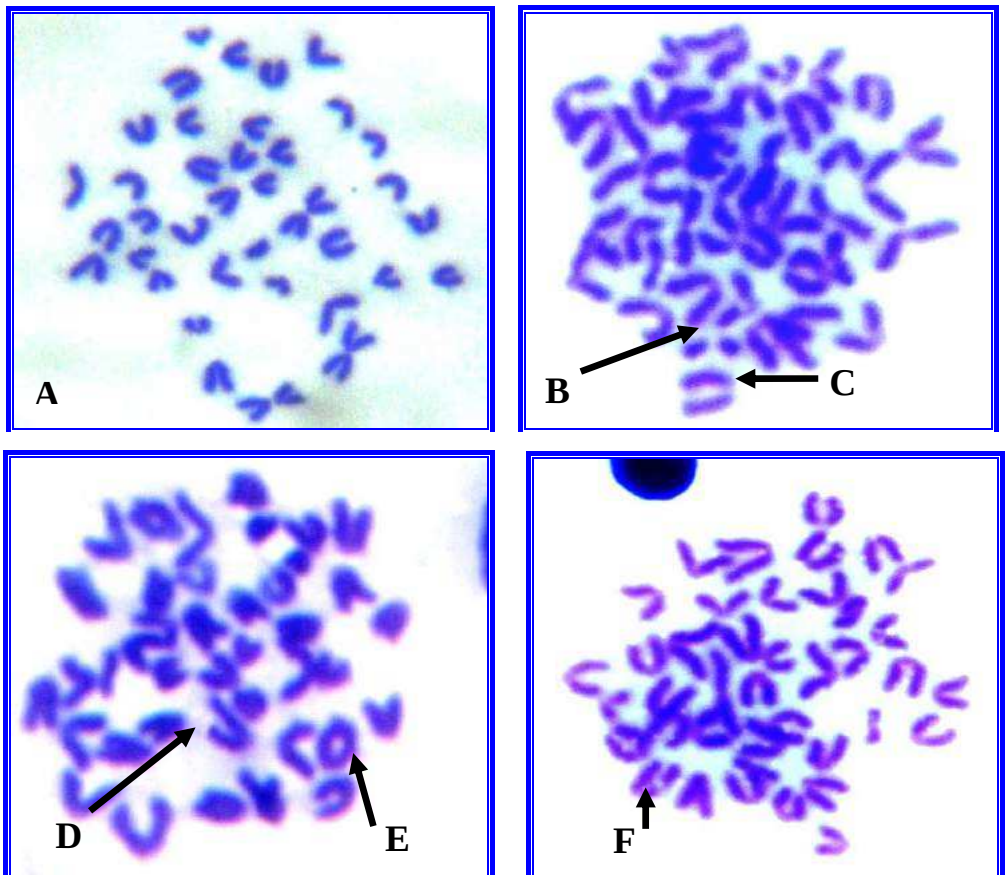

Figure (1): Different types of chromosomal aberrations induced by miller herbicide in bone marrow cells of Swiss albino males mice **(100x).** **A-** normal chromosomes; **B-** centromeric break; **C-** centromeric gap; **D-** Deletion **E-** Ring Chromosome; **F-** Chromatid gap.

Effects of Miller on sperms of mice

The data in tables (2A and B) represent the effects of miller on the sperms of Swiss albino male mice. The values of these traits in tables (2 A and B) were the number of abnormal sperms counted from total of 1000 sperms. From these data, it is obvious that there is a high significant difference in all types of sperm abnormalities listed in the tables due to the effects of the treatments when compared to control. The total number of abnormal sperms increased constantly with increase of miller doses. The highest total number of abnormal sperms was in D3 = 120.7. The highest mean value of abnormal sperms head in treated animals (Table 2.A) compared to other types of head abnormalities was 27.00±2.43 in amorphous head sperm. The highest mean value of abnormal sperms tail (Table 2.B) compared to other types of tail abnormalities was 30.80±1.90 in bent mid piece defect. The least affected trait was the sperms with swollen head with value of 1.40±0.22 in D0 treatment.

There were a high significant differences ($p<0.01$) between all doses due to the effects of periods, as the total number of affected sperm in the first period was 136.75 increased to 214.20 in the second period. The most affected traits by the periods were sperms with bent mid piece defect and sperms with coiled tail, while the least affected trait was the sperms with double tail (Table 2 B).

There was a significant differences ($p<0.01$) between treatments due to the interactions between doses and periods (Tables 2 A and B). The non affected traits were Sperms with Banana head, Sperms with Double heads and Sperms with Double tail. The highest value of total abnormal sperm was in D3P2 with value of 142.4, while the least interaction was in D0P1 with total abnormal sperms 38. The most interaction between the periods and doses was in D3P2 with value of 35.40±1.72 scored in sperms with bent mid piece defect, while no interactions in D0P1 for sperms with double tail.

Most types of sperms abnormalities in males of albino mice induced by Miller herbicide are shown in Figure (2).

Table (2.A): Mean values of different abnormalities in sperms heads of Swiss albino mice induced by miller

Factors		Sperms Head Abnormalities						
		Hookless sperms	Sperms with Swollen head	sperms with amorphous head	Sperms with Defected hook	Sperms with Banana head	Sperms with Double heads	Total of sperms with abnormal heads
Doses	D0(control)	3.20±0.48c	1.40±0.22c	9.30±0.55c	2.10±0.31c	3.50±0.34c	0.20±0.13	19.7
	D1	10.60± 0.92b	10.20±0.72b	18.80±1.48b	5.90±0.67b	5.90±0.58b	0.20±0.13	51.6
	D2	11.10±0.87b	9.90±0.91b	20.10±1.44b	6.60±0.66b	7.60±0.54ab	0.10±0.10	55.4
	D3	16.40±1.43a	14.00±0.64a	27.00±2.43a	9.40±0.68a	9.00±0.57a	0.30±0.15	76.1
	significant	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	N.S	
Periods	P1	9.250±0.99b	8.10±1.14b	15.25±1.03b	5.25±0.55b	5.80±0.50b	0.150±0.08	43.8
	P2	11.40±1.46a	9.65±1.14a	22.35±2.07a	6.75±0.83a	7.20±0.62a	0.250±0.09	57.6
	significant	p<0.01	p<0.05	p<0.01	p<0.01	p<0.01	N.S	
Interactions(Doses and Periods)	D0P1	3.00±0.70c	1.20±0.20e	9.00±0.70e	2.20±0.58	3.60±0.50	0.20±0.200	19.2
	D0P2	3.40±0.74c	1.60±0.40e	9.60±0.92e	2.00±0.31	3.40±0.50	0.20±0.200	20.2
	D1P1	12.00±1.30b	9.40±1.24cd	15.20±1.06d	5.00±0.70	4.80±0.66	0.20±0.200	46.6
	D1P2	9.20±1.06b	11.00±0.70bc	22.40±1.53b	6.80±1.06	7.00±0.70	0.20±0.200	56.6
	D2P1	9.00±0.70b	7.60±0.60d	16.60±1.20cd	6.00±0.70	6.80±0.86	0.00±0.00	46
	D2P2	13.20±0.86b	12.20±0.86ab	23.60±1.32b	7.20±1.15	8.40±0.50	0.20±0.200	64.8
	D3P1	13.00±1.00b	14.20±1.06a	20.20±0.86bc	7.80±0.58	8.00±0.70	0.20±0.200	63.4
	D3P2	19.80±1.59a	13.80±0.86a	33.80±1.65a	11.00±0.70	10.00±0.70	0.40±0.24	88.8
	significant	p<0.01	p<0.05	p<0.01	N.S	N.S	N.S	

Similar letters in each column refer to non significant difference while different letters refer to significant difference between them.
N.S=significant differences

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Table (2.B): Mean values of different abnormalities in sperms tail of Swiss albino mice induced by miller

Factors		Sperms Tail Abnormalities				
		Sperms with Double tail	Sperms with bent midpiece defect	Sperms with Coiled tail	Total of sperms With abnormal tails	Total no. of abnormal sperm
Doses	D0(control)	0.10±0.10b	16.30±0.81b	2.30±0.30c	18.7	38.4
	D1	0.50±0.22ab	30.10±2.34a	7.50±0.52b	38.1	89.7
	D2	0.40±0.16ab	28.30±1.94a	7.80±0.66b	36.5	91.9
	D3	1.10±0.31a	30.80±1.90a	12.70±1.36a	44.6	120.7
	significant	p<0.01	p<0.01	p<0.01		
Periods	P1	0.200±0.09b	22.550±1.07b	6.000±0.65b	28.75	72.55
	P2	0.850±0.19a	30.200±2.03a	9.150±1.17a	40.2	97.8
	significant	p<0.01	p<0.01	p<0.01		
Interactions(Doses and Periods)	D0P1	0.00±0.00	16.80±1.24c	2.00±0.31d	18.8	38
	D0P2	0.20±0.20	15.80±1.15c	2.60±0.50d	18.6	38.8
	D1P1	0.20±0.20	23.80±1.93b	6.80± 0.80bc	30.8	77.4
	D1P2	0.80±0.37	36.40±1.07a	8.20±0.58bc	45.4	102
	D2P1	0.20±0.20	23.40±1.36b	6.20±0.66c	29.8	75.8
	D2P2	0.60±0.24	33.20±1.77a	9.40±0.50b	43.2	108
	D3P1	0.40±0.24	26.20±1.65b	9.00±0.70b	35.6	99
	D3P2	1.80±0.37	35.40±1.72a	16.40±1.02a	53.6	142.4
	significant	N.S	p<0.01	p<0.01		

Similar letters in each column refer to non significant difference while different letters refer to significant difference between them. N.S :Non significant.

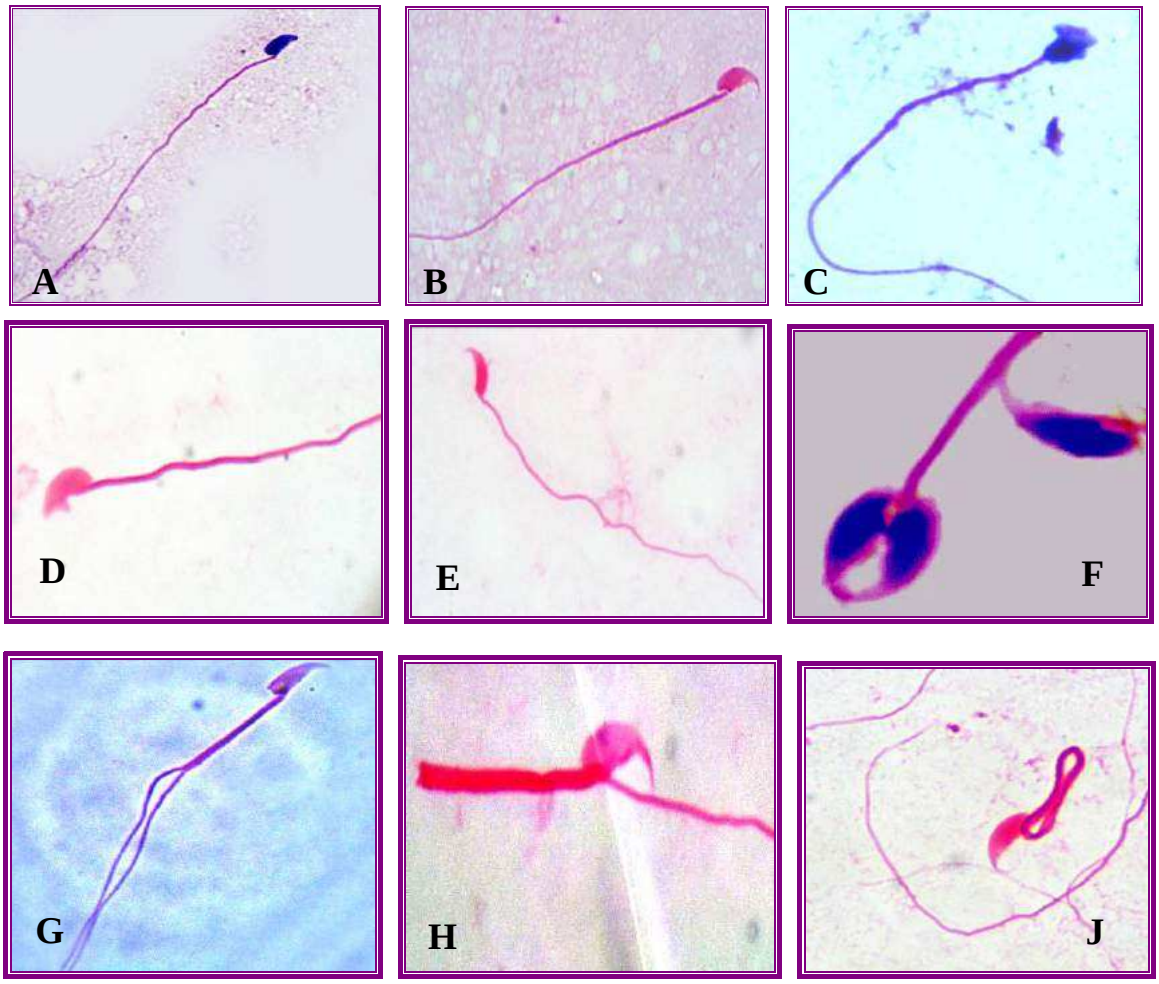

Figure (2): Different types of mice sperms abnormalities induced by miller herbicide (100x). A- hookless sperms; B- swollen head sperm; C-sperm with amorphous head; D-sperms with defected hook; E- sperms with banana head; F- Sperm Double heads; G- Sperms with Double tail; H- Sperms with bent midpiece defect; J- Sperms with Coiled tail.

Discussion

Effects of Miller on chromosome of bone marrow cells of mice

Miller or glyphosate, being one of the most commonly used herbicides, has been extensively studied over the last 20 years. Literature on cytotoxic and genotoxic activity glyphosate is abundant but it has included a lot of controversial data (Buffin and Jewell, 2001). In the present study, the genotoxicity of miller was revaluated. The experimental data revealed significant differences between the doses in their effects on all types of chromosomal aberrations except the dicentric chromosome. Percentage of chromosomal aberrations increased with increasing dose concentration. These results are smiler to those reported by Prasad *et al.*, (2009), in which glyphosate were administered by a single intraperitoneal dose (25 and 50 mg/kg b.wt) to males of albino mice and they found that glyphosate significantly increase chromosomal aberrations in both treatment and time compared to the control. They attributed it either to induction of DNA lesions or interference of glyphosate with DNA repair. Peluso *et al.*,(1998) also reported a dramatic increase in the number of oxidized guanine, 8-hydroxylguanine (8-OHdG), residues in the DNA of liver cells isolated from mice treated with Glyphosate which also may be the reason of chromosomal aberrations in bone marrow cells of mice as observed in the current study. Concurrently, Bhunya and Behera (1988) observed similar increase in chromosomal aberrations in bone marrow cells of Swiss albino mice when they used Monocrotophose pesticide in their experiments. These results also agree with those reported by Taha, (2000) who showed that exposure to granstar herbicide induce chromosomal aberrations in all doses and

periods. As well same results were obtained by Jha *et al*., (2002) who explained that increasing dose of carbazole lead to increase the frequency of some chromosomal aberrations per cells in bone marrow cells of albino mice.

The present study show significant differences between periods in their effects on the increase of chromosomal aberrations except for dicentric chromosome aberration. These results are supported by Sulaiman, (2000) who observed that chromosomal aberration is dependent on the duration of the study when he studies the genetic and histological effects of cigarette smoke on albino mice. Similar results were also obtained by Holden *et al*., (1973) who showed that increasing exposure time would increase the rate of chromosomal aberrations in bone marrow cells of CD-1 albino mice treated with 6-mercapto purine. In contrast to these results Kourakis *et al*., (1992) observed no positive correlation between the frequency of chromosomal aberrations and the duration of exposure in peripheral blood lymphocytes of workers occupationally exposed to mixture of pesticides. The present study revealed that some types of aberration increased with increasing exposure time, which might be due to that some cells exposed to harmful effects at any phases of the cell cycle, also the chromosomal density in these stages are the highest.

The interactions between doses and periods and their effects on chromosomal aberrations show no significant effects on all types of chromosomal aberrations except for ring chromosome aberration.

Effects of miller on sperms of mice

The result of the current study shows that different doses of Miller caused highly significant differences in all types of sperm abnormalities except double head sperms abnormality. The third dose was the most effective in causing most types of sperms abnormalities. These results are similar to those reported by El-Nahas *et al.* (1989) who explained that Curacron (an organophosphorus pesticide) caused different morphological sperm abnormalities which increased significantly after treatment with Curacron. The increase was dose-dependent. Similarly, Mathew *et al.* (1992) observed that Methyl-parathion showed a dose related increase in the percentage of sperm abnormalities. The current results also agree with the findings reported by Yousef *et al*. (1995) who explained that glyphosate showed decline in body weight, libido, ejaculation volume, sperm concentration and increases in the abnormal and dead sperms. Hurtado de Catalfo Graciela *et al*. (2011) as well noticed alterations in spermatozoa morphology and in plasma membrane integrity when they tested dimethoate, glyphosate and zineb. Radioimmuno assay analyses (RIA) demonstrated androgenic hormone imbalance in plasma and testes. The acrosome reaction was also altered. Free thiols (positively correlated with DNA denaturation) and fructose levels were elevated in seminal vesicles from treated rats. Taking into account the low doses of pesticides that provoke these alterations, it was assumed that the environmental pollution may play a key role as a causative factor for sperms abnormalities. These results are another support to the present study. Wyrobek *et al.* (1975) mentioned that the chromosomal aberrations may cause abnormal sperm morphology in a number of ways. One possibility is that the presence of a translocated chromosome within the germ cell will lead to the malformation of the sperm head. A second possibility is that chromosomal imbalance, i.e., aneuploidy, duplications or deficiencies, within the spermatid or haploid cells causes abnormalities in shape. These notes may explain the formation of abnormal sperms in the current study.

Periods showed significant effects on all types of sperms abnormalities except double head sperm abnormality. Furthermore, the results showed that the second period was highly effective in inducing most types of sperm abnormalities. Similar results were observed by Taha, (2000) who explained that the periods had significant effects on sperm abnormalities when he studied the effect of different periods on sperm abnormalities in male mice treated with the herbicide granstar.

In this study, the interaction between doses and periods and their effects on sperm abnormalities revealed significant differences in most types of sperm abnormalities. Since the process of spermatogenesis is genetically controlled, the abnormalities in sperms reflect the abnormalities in chromosomes of spermatogenic cells. The spermatogenesis is a continuous process and the inducing agents may act at various steps of spermiogenesis. This may be the reason of the inconsistence response to glyphosate doses and periods which led to significant differences due to the interaction

between doses and periods in most of sperm parameters.

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NUCLEOTIDE SEQUENCE ANALYSIS OF METHICILLIN RESISTANCE *STAPHYLOCOCCUS AUREUS* IN KURDISTAN REGION-IRAQ

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Abstract

In this study a total of 241 clinically important specimens were collected from three hospitals in Kurdistan region; from which 64 isolates of *S. aureus* were isolated representing 26.5% of the total samples. The molecular confirmation of *S. aureus* isolates were done by PCR using nuc primer as a species-specific primer producing 280bp DNA fragment for *nuc* gene. 31 of these isolates were resistance to methicillin representing 48.4% of total isolates. Resistance to Methicillin was confirmed by using mecA primer producing 310bp DNA fragment for *mecA* gene. Three isolates (SaDu5, SaEr17 and SaSul24) were subjected to partial DNA sequencing of the *mecA* gene to assess the relation of Kurdistan isolates with other global sequences from GenBank. Sequence analysis showed that the sequence identity of all three *mecA* genes among the Kurdistan isolates was ranged between 97.5-98.5%, whereas the sequence identity among the Kurdistan isolates and other global isolates selected from GenBank was ranged between 92.6-96.1%. The sequenced product of *S. aureus mecA* genes from Kurdistan found similarities and showed partial homology with *S. aureus* strains (UK/NTCC124, Turkey/HM5, South Korea/ YSSA11 and India/TN/CN/1/12) penicillin binding protein 2a *mecA* gene, partial cds, which is available in public databases. The phylogenetic tree of the aligned sequences was produced by using MEGA5 Beta version 6.1 online software, and showed a typical relatedness among Kurdistan isolates and GenBank reference strains. This study will gives insights in to the molecular diagnosis of *S. aureus* and to find better synthetic drugs or organic to control expression of antibiotic genes in different pathogenic organism in general and in particular MRSA strains.

Key words: MRSA, *mec*A gene, DNA Sequencing.

Introduction:

Staphylococcus aureus is one of the most significant and serious human pathogens, which is known as a threat to human health for more than a century. This pathogen is responsible for a wide range of diseases, causing both nosocomial and community-acquired infections (Askari *et al*., 2012). Acquisition of resistance to several antimicrobial agents by this pathogen has been a major problem for the treatment of infections in the hospital and the community settings. Resistance to β-lactam antibiotics such as methicillin and oxacillin, is named "methicillin resistance" and is considered of great importance since the multidrugresistant phenotype of MRSA strains and their intrinsic β-lactam resistance make them difficult and costly to treat (Paradisi *et. al.*, 2001). Most strains of Methicillin Resistance *S. aureus* (MRSA) are also resistant to other β-lactam antibiotics, with the exception of glycopeptide antibiotics (Tiwari *et. al.*, 2006). Methicillin resistance is mediated by the presence of PBP-2a which is highly conserved among staphylococci and is encoded by the chromosomal gene *mecA* (Malik *et al*., 2006). *mecA* is located on a genetic element called the staphylococcal cassette chromosome (SCC) in *Staphylococcus aureus*. SCC*mec* is a group of mobile DNA fragments of about 21 to 67 kb that is integrated into the chromosome of methicillin resistant *S. aureus* at a unique site (*attBscc*) located near the *S. aureus* origin of replication (Hanssen *et. al*., 2004). The incidence of MRSA has become increasingly prevalent in the world since it first reported. Subsequently, the occurrence of MRSA particularly in hospitalized patients has increased steadily and nosocomial infections caused by such strains have become a serious problem worldwide. Therefore, rapid and accurate identification of MRSA is essential (Terry *et. al*., 2011). Improved strain characterization would improve our understanding of the epidemiology of this pathogen and will allow the development of a rapid assay for monitoring and controlling of MRSA. In view of this, the detection of *mecA* gene has been used as an alternative way of detecting or confirming MRSA either by use of DNA probe, commercially available fluorescence test, latex agglutination test, and PCR (Ieven *et al*., 1995, Cavassini *et. al*., 1999 and Rohrer *et. al*., 2001). Molecular typing techniques are widely applied in studies of *S. aureus* epidemiology, especially MRSA, with the aim of distinguishing isolates that are epidemiologically related from those unrelated. *S. aureus* outbreaks in hospitals are frequently

considered as short duration events of localized epidemiology and, in these situations, most of the molecular typing methods are able to distinguish the isolates that belong to the outbreak (Shopsin and Kreiswirth, 2001).

The rapidly expanding number of sequenced microbial genomes has served as a catalyst for the development of a variety of molecular typing approaches that focus on either single or multiple chromosomal loci. Single loci sequence typing (SLST) Sequence data for specific loci (genes for virulence, pathogenicity, drug resistance …etc.) from different strains of the same species have revealed variability in a specific gene, such as single-nucleotide polymorphisms and areas with repetitive sequence that demonstrate potential for epidemiologic application. SLST shows potential for a typing and clearly represents an important and promising sequenced-based approach to epidemiologic analysis (Singh *et. al*., 2006). Thus the objectives of this study were to molecular identification of *S. aureus* by using nuc primer as a species-specific primer and to determine the nucleotide sequence of *mecA* gene. Furthermore, analysis and determine the phylogenetic diversity and nucleotide sequence comparison of *S. aureus* isolates depending on the sequence of *mecA* gene in Kurdistan Region-Iraq.

Materials and Methods:

Bacterial isolation

Isolates of *S. aureus* used in this study were collected from Azadi, Dr. Khalid and Rizgary General Hospitals in Duhok, Koya and Erbil cities respectively in Kurdistan Region-Iraq. These samples were taken from patients, healthcare staffs and hospital environment. Basing on the source of infections, the isolates were obtained from: urine, nose, throat, wounds, burns, skin infections and hospital environments. These isolates were confirmed at molecular level as *S. aureus* by species-specific PCR technique using nuc primer.

Antimicrobial Susceptibility Testing

Disk diffusion testing was performed as recommended for staphylococci by Clinical and Laboratory Standards Institute (CLSI, 2012). Strains were considered susceptible to oxacillin if the zone of inhibition around the 1µg disks was >13 mm, intermediate if 11-12 mm, and resistant if the zone was < 10 mm.

Oligonucleotides sequences

Oligonucleotides of *nuc* gene as species-specific primer and *mec*A for Methicillin Resistant gene detection were used in this study provided by MWG Company, Germany (Table: 2) (Merza 2009).

PCR amplification:

The PCR amplification was carried out in 50-µl reaction mixture volumes containing 4µl of genomic DNA, 2 µl of each primer (50 pmol/µl), 5 µl of 10× PCR buffer (100mM Tris-base pH8.3, 500mM KCl, 20mM $MgCl_2$ and 0.001 gelatin), 5 µl of 100 mM deoxynucleoside triphosphates, and 2 units of *Taq* DNA polymerase (Gold Taq). DNA amplification was carried out in Applied Biosystem (USA) thermocycler, with the following thermal cycling profile for nuc gene: initial denaturation at 94°C for 4 min was followed by 30 cycles as follows: denaturation at 94°C for 45sec, annealing at 50°C for 45sec, and DNA extension at 72°C for 1 min. The reaction was achieved with a final extension at 72°C for 2 min. (Tiwari *et. al*., 2006).

The reaction condition for detection *mecA* gene was programmed with the initial denaturation, 4 min at 94°C; 30 cycles with a 45-s denaturation step at 94°C, a 45-s annealing step at 56°C and a 30-s extension step at 72°C and 2 min extension step at 72°C (Geha *et. al*., 1994). Five microliters of the amplified PCR products were used for detection of amplified products by electrophoresis in a 2.0% agarose gel and visualization with UV illumination after staining with ethidium bromide for 15 min. Standard DNA ladder 1500-100bp promega Company (USA) were included in each run.

DNA sequencing:

The amplification products were purified by using Illustra ™ GFX ™ PCR DNA and GeL Band Purification Kit (GE Healthcare Company, UK) following the manufacturers protocol before being used in a sequencing reaction. Sequencing was carried out on each DNA strand with BigDye Terminator Ready Reaction Mix v3.1 (AB Biosystems, HITACHI) by using the same primer set for *mecA* gene under the following conditions: initial denaturation at 96°C for 1 min, 30 cycles of 10 sec at 96°C, 5 sec at 51°C and 2 min at 60°C. Unincorporated dye terminators were removed by precipitation with 95% alcohol. The reaction products were separated and detected on an Applied Biosystem

genetic analyser 3130 (AB Biosystems) using a standard sequencing module with a Performance Optimised Polymer 7 (POP7) and 36 cm capillary array.

Nucleotide sequence analysis:

The sequences were compared to the nucleotide sequence database from the National Center for Biotechnology Information (NCBI) using the BLAST tool (http://www.ncbi.nlm.nih.gov/sites/entrez). Restriction map was performed by using ChromasPro (version 1.5) online software. Multiple sequence alignments were performed by using ClustalW (http://align.genome.jp) software (www.ebi.ac.uk/clustalw). Phylogenetic tree was performed by using MEGA5 Beta version 6.1 online software (www.megasoftware.net).

GenBank accession numbers:

GenBank accession numbers of *S. aureus* sequences used in the analysis are: Turkey/HM5 (EU790490.1), Iran/M795 (GU301104.1), Egypt/1 (EF600988.1), India/TN/CN/1/12 (KC243783.1), South Korea/ YSSA11 (EF596937.1) and UK/NTCC124 (HQ686324.1).

Results and Discussions

In this study a total of 241 clinically important specimens were collected; from which 64 isolates of *S. aureus* were isolated representing 26.5% of the total samples. These samples obtained from three hospitals (Dr. Khalid General Hospital in Koya city, Rizgary General Hospital in Erbil city and Azadi General Hospital in Duhok city) in three districts in Kurdistan-Iraq. These samples collected from patients, healthcare staffs and hospital environment in hospitals mentioned above (Table: 1). Isolates were obtained from urine, nose, throat, wounds, burns, skin infections and Hospital environments. Healthcare staffs included physicians, nurses and workers. Hospital environment samples were obtained from different sites in surgical, intensive care, burn, emergency, laboratory units and delivery ward. The molecular confirmation of *S. aureus* isolates was done by using nuc primer as a species-specific primer for *S. aureus*. The results showed that all isolates were amplified and producing 280bp DNA fragment for *nuc* gene as illustrated in (Figure: 1.a). All 64 isolates of *S. aureus* were searched for methicillin resistant by using Oxacillin (1μg) disc diffusion method according to the Clinical and Laboratory Standards Institute guidelines (Antimicrobial Susceptibility Testing Standards) (CLSI, 2012). The results revealed that the prevalence of methicillin resistant *S. aureus* in Kurdistan was very high, which found that 31 of these isolates were resistance to methicillin representing 48.4% of total isolates as illustrated in (Table:2). The high prevalence of MRSA immerged in our hospitals may reflect failures in strategies to control infection and/or differences in antibiotic use, the poor hygienic conditions of the patients. Resistance to methicillin is mediated by the acquisition of penicillin binding protein PBP-2a encoded by the *mec*A gene, which exhibits a low affinity for β-lactam antibiotics (Davoodi *et al*., 2012). Resistance to Methicillin was confirmed by using mecA primer producing 310bp DNA fragment for *mecA* gene as shown in (Figure: 1.b).

Three methicillin-resistant staphylococcal isolates (SaDu5, SaEr17 and SaSul24) were subjected to partial DNA sequencing of the *mecA* gene to assess the relation of Kurdistan isolates with other global sequences from GenBank and to find similarity between them for cataloging mutation patterns and to provide useful strain resolution. Sequence analysis shows that the sequence identity of all three *mecA* genes among Kurdistan isolates was ranged between 97.5-98.5%, whereas the sequence identity among Kurdistan isolates and other global isolates selected from GenBank was ranged between 92.6-96.1% (Table: 3). Identical sequences among Kurdistan isolates and other global isolates obtained from the National Center for Biotechnology Information (NCBI) database, supporting the hypothesis that horizontal transfer plays an important role in the dissemination of the *mecA* gene in the *S. aureus* population. Only a few restriction enzymes can be used to differentiate between these closely related isolates (Fig. 2), these enzymes can be used for restriction fragment length polymorphism (RFLP) to identify and differentiate between them. Multiple sequence alignment of three Kurdistan isolates with other 6 global isolates from GenBank has been performed using ClustalW 2.0.12 multiple sequence alignment software (Fig. 3). The sequenced product of *S. aureus mecA* genes from Kurdistan when compared with *mecA* gene of *S. aureus* strain from GenBank found

similarities and showed partial homology with *S. aureus* strains (UK/NTCC124, Turkey/HM5, South Korea/ YSSA11 and India/TN/CN/1/12) penicillin binding protein 2a *mecA* gene, partial cds, which is available in public databases. The phylogenetic tree of the aligned sequences was also produced using MEGA5 Beta version 6.1 online software (www.megasoftware.net), and showed a typical relatedness among Kurdistan isolates and GenBank reference strains (Fig. 4). As a result, the structure of the same gene reported by different labs can be different! Multiple sequence alignment and phylogenetic tree analysis separate the isolates into different groups which are genotypically related to each other. DNA sequence analysis of the *mecA* gene provides an unambiguous, portable dataset that simplifies information sharing between laboratories and facilitates creating a large scale database for studying global and local epidemiology. Amplification of specific gene and sequencing of *mecA* gene gives insight into pharmaceutical aspects to design new effective drugs for treatment of methicillin resistance *S. aureus*. In conclusion the current study of isolation, molecular identification and sequencing of the *mecA* gene from MRSA strain will give insight in to the molecular diagnosis of *S. aureus* and to find better synthetic drugs (analogs) or organic molecules by computational biologist in pharmaceutical companies to control expression of antibiotic genes in different pathogenic organism in general and in particular MRSA strains.

Table 1: Source, location, number of isolates and methicillin resistant isolates.

Hospital	Location	Samples	Isolates	MRSA
Dr. Khalid	**Koya**	62	16	7
Rizgary	**Erbil**	93	27	13
Azadi	**Duhok**	86	21	11
Total		241	64	31

Table 2: Oligonucleotides used in this study.

Primer name	Target gene	Forward 5'----------3'	Reverse5'----------3'	Size(bp.)
mecA	PBP2a	GTAGAAATGACTGAACGT CCGATGA	CCAATTCCACATTGTTTCG GTCTAA	310 (Merza 2009)
nuc	Nuclease	GCGATTGATGGTGATACG GTT	AGCCAAGCCTTGACGAACT AAAGC	280 (Merza 2009)

Table 3: Sequence Identity Matrix of *S. aureus mecA* gene partial sequence.

Seq->	Kurdistan/ Du5	Kurdistan/ Er17	Kurdistan/ Sul24	Turkey /HM5	Iran/ M795	Egypt/1	India/TN/CN/1/12	S.Korea/YSSA11	UK/NTCC12493
Kurdistan/Du5	ID	0.975	0.985	0.961	0.957	0.933	0.954	0.961	0.961
Kurdistan/Er17	0.975	ID	0.971	0.957	0.961	0.926	0.943	0.957	0.957
Kurdistan/Sul24	0.985	0.971	ID	0.961	0.957	0.933	0.950	0.961	0.961
Turkey/HM5	0.961	0.957	0.961	ID	0.996	0.939	0.967	1.000	1.000
Iran/M795	0.957	0.961	0.957	0.996	ID	0.935	0.964	0.996	0.996
Egypt/1	0.933	0.926	0.933	0.939	0.935	ID	0.967	0.939	0.939
India/TN/CN/1/12	0.954	0.943	0.950	0.967	0.964	0.967	ID	0.967	0.967
S.Korea/YSSA11	0.961	0.957	0.961	1.000	0.996	0.939	0.967	ID	1.000
UK/NTCC12493	0.961	0.957	0.961	1.000	0.996	0.939	0.967	1.000	ID

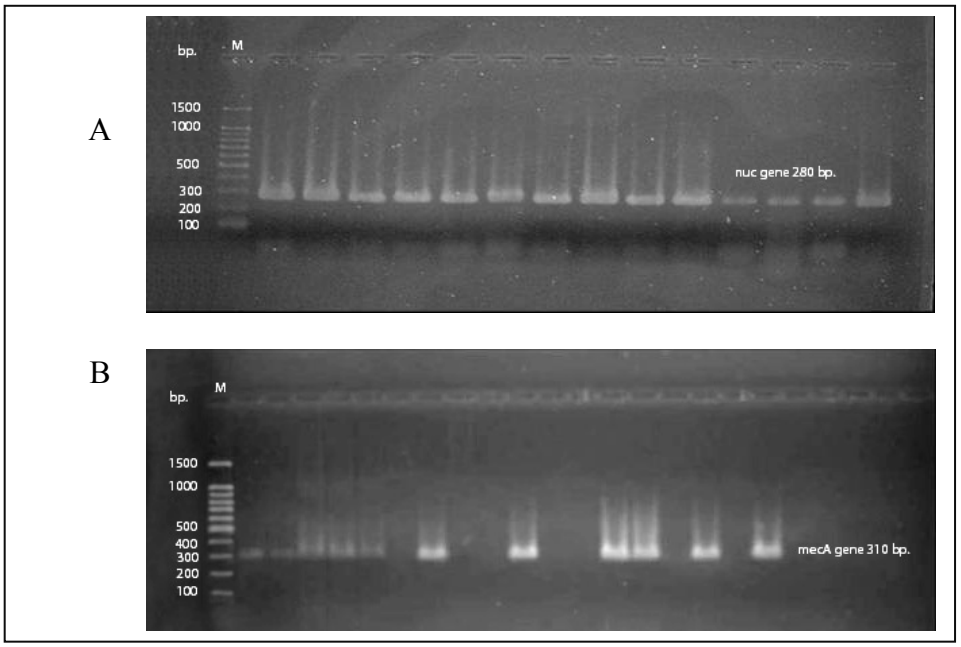

Figure (1): A. Illustrates the gel electrophoresis of amplified DNA fragments generated by species-specific PCR amplification (nuc primer), and (**B**) illustrates the amplified DNA fragments for detection of *mecA* gene.

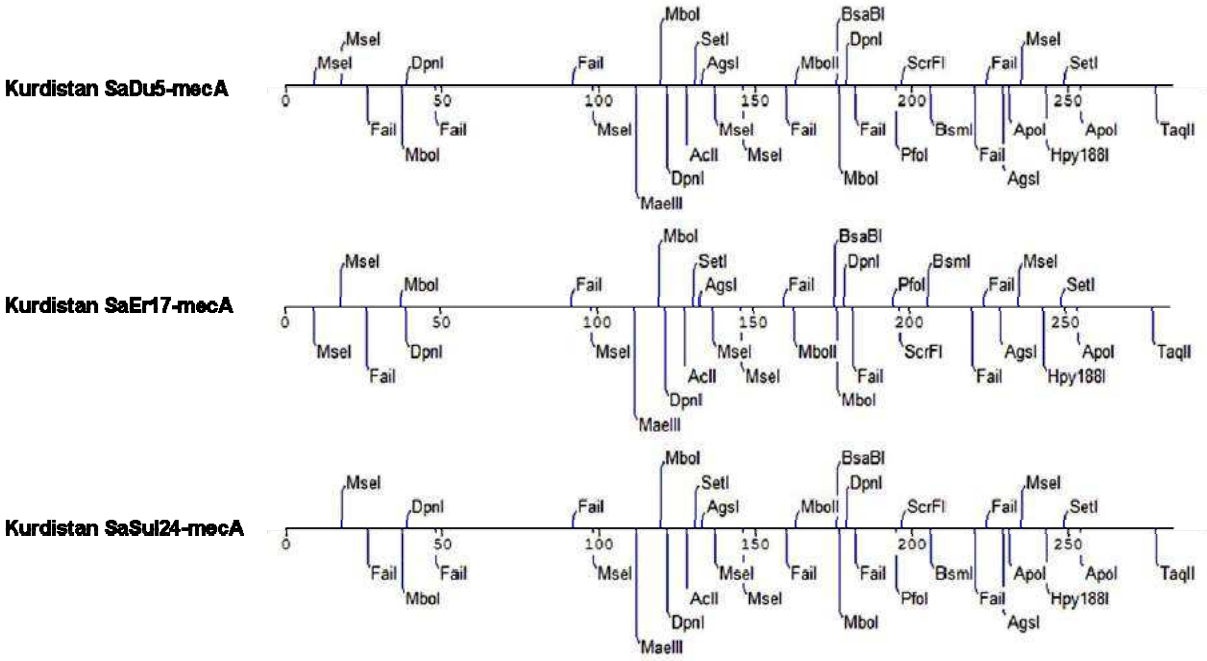

Figure: 2. Restriction map of Kurdistan isolates of *S. aureus* (*mecA*) gene partial sequence that show the restriction enzymes which can be used to differentiate between these isolates. The process was done by using ChromasPro (version 1.5) online software.

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                                10        20        30        40        50        60        70        80
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|...
Kurdistan SaDu5-mecA            AAATAATTTTAAGGCGTTTAAAGATATAAACATTCAGGATCGTAAATATAAAAAAAAGTATCTAAAAATAAAAAACGAGTAGATGCTC
Kurdistan SaEr17-mecA           AAAAAGATTTTAAGCCGTTTAAAAATATAAACATTCAGGATCGTAAAAATAAAAAAAAGTATCTAAAAATAAAAAACGAGTAGATGCTC
Kurdistan SaSul24-mecA          AAAAGTTTTTAGGGCGTTTAAAGATATAAACATTCAGGATCGTAAATATAAAAAAAAGTATCTAAAAATAAAAAACGAGTAGATGCTC
Turkey SaHM5-mecA               TATAATAGTTTAGGCGTT-AAAGATATAAACATTCAGGATCGTAAA-ATAAAAAAA-GTATCTAAAAATAAAAAACGAGTAGATGCTC
Iran SaM795-mecA                TATAATAGTTTAGGCGTT-AAAGATATAAACATTCAGGATCGTAAA-ATAAAAAAA-GTATCTAAAAATAAAAAACGAGTAGATGCTC
Egypt Sa-mecA                   ---------------GTT-AA-GATATAAACATTCAGGATCGTAAA-ATAAAAAAA-GTATCTAAAAATAAAAAACGAGTAGATGCTC
India SaTN/CN/1/12-mecA         --------TTTAGGCGTT-AAAGATATAAACATTCAGGATCGTAAA-ATAAAAAAA-GTATCTAAAAATAAAAAACGAGTAGATGCTC
S.Korea SaYSSA11-mecA           TATAATAGTTTAGGCGTT-AAAGATATAAACATTCAGGATCGTAAA-ATAAAAAAA-GTATCTAAAAATAAAAAACGAGTAGATGCTC
UK SaNTCC12493-mecA)            TATAATAGTTTAGGCGTT-AAAGATATAAACATTCAGGATCGTAAA-ATAAAAAAA-GTATCTAAAAATAAAAAACGAGTAGATGCTC

                                110       120       130       140       150       160       170       180
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|...
Kurdistan SaDu5-mecA            AAACAAACTACGGTAACATTGATCGCAACGTTCAATTTAATTTTGTTAAAGAAGATGGTATGTGGAAGTTAGATTGGGATCATAGCGT
Kurdistan SaEr17-mecA           AAACAAACTACGGTAACATTGATCGCAACGTTCAATTTAATTTTGTTAAAGAAGATGGTATGTGGAAGTTAGATTGGGATCATAGCGT
Kurdistan SaSul24-mecA          AAACAAACTACGGTAACATTGATCGCAACGTTCAATTTAATTTTGTTAAAGAAGATGGTATGTGGAAGTTAGATTGGGATCATAGCGT
Turkey SaHM5-mecA               AAACAAACTACGGTAACATTGATCGCAACGTTCAATTTAATTTTGTTAAAGAAGATGGTATGTGGAAGTTAGATTGGGATCATAGCGT
Iran SaM795-mecA                AAACAAACTACGGTAACATTGATCGCAACGTTCAATTTAATTTTGTTAAAGAAGATGGTATGTGGAAGTTAGATTGGGATCATAGCGT
Egypt Sa-mecA                   AAACAAACTACGGTAACATTGATCGCAACGTTCAATTTAATTTTGTTAAAGAAGATGGTATGTGGAAGTTAGATTGGGATCATAGCGT
India SaTN/CN/1/12-mecA         AAACAAACTACGGTAACATTGATCGCAACGTTCAATTTAATTTTGTTAAAGAAGATGGTATGTGGAAGTTAGATTGGGATCATAGCGT
S.Korea SaYSSA11-mecA           AAACAAACTACGGTAACATTGATCGCAACGTTCAATTTAATTTTGTTAAAGAAGATGGTATGTGGAAGTTAGATTGGGATCATAGCGT
UK SaNTCC12493-mecA)            AAACAAACTACGGTAACATTGATCGCAACGTTCAATTTAATTTTGTTAAAGAAGATGGTATGTGGAAGTTAGATTGGGATCATAGCGT

                                210       220       230       240       250       260       270       280
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....
Kurdistan SaDu5-mecA            AATGCAGAAAGACCAAAGCATACATATTGAAAATTTAAAATCAGAACGTGGTAAAATTTTAGACCGAAACAATGTGGAATTGGA
Kurdistan SaEr17-mecA           AATGCAGAAAGACCAAAGCATACATATTGAAAAATTAAAATCAGAACGTGGTAAAATTTTAGACCGAAACAATGTGGAATTGGA
Kurdistan SaSul24-mecA          AATGCAGAAAGACCAAAGCATACATATTGAAAATTTAAAATCAGAACGTGGTAAAATTTTAGACCGAAACAATGTGGAATTGGA
Turkey SaHM5-mecA               AATGCAGAAAGACCAAAGCATACATATTGAAAATTTAAAATCAGAACGTGGTAAAATTTTAGACCGAAACAATGTGGAATTGGC
Iran SaM795-mecA                AATGCAGAAAGACCAAAGCATACATATTGAAAAATTAAAATCAGAACGTGGTAAAATTTTAGACCGAAACAATGTGGAATTGGC
Egypt Sa-mecA                   AATGCAGAAAGACCAAAGCATACATATTGAAAATTTAAAATCAGAACGTGGTAAAATTTTAGACCGAAACAATGTGGAATTGGA
India SaTN/CN/1/12-mecA         AATGCAGAAAGACCAAAGCATACATATTGAAAATTTAAAATCAGAACGTGGTAAAATTTTAGACCGAAACAATGTGGAATTGG-
S.Korea SaYSSA11-mecA           AATGCAGAAAGACCAAAGCATACATATTGAAAATTTAAAATCAGAACGTGGTAAAATTTTAGACCGAAACAATGTGGAATTGGC
UK SaNTCC12493-mecA)            AATGCAGAAAGACCAAAGCATACATATTGAAAATTTAAAATCAGAACGTGGTAAAATTTTAGACCGAAACAATGTGGAATTGGC
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Figure: 3. Multiple sequence alignment of the sequenced part of *S. aureus* (mecA) gene of Kurdistan isolates with other selected isolates from GenBank using (CLUSTAL 2.0.12 multiple sequence alignment) showing partial homology to *S. aureus* strains penicillin binding protein 2a (mecA) gene, partial cds. Gaps indicate in (-).

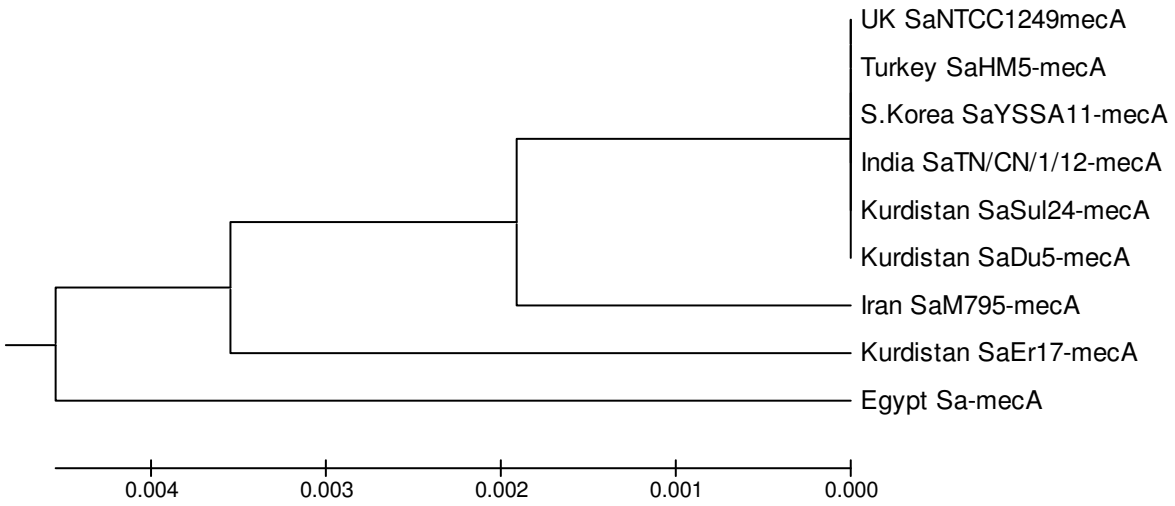

Figure: 4. Phylogenetic tree of Kurdistan isolates and other selected strains from GenBank representing different geographic regions shows the relationship among the *mecA* gene sequence of Kurdistan isolates with other selected isolates from GenBank.

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شیتەڵکردنی زنجیرەی ترشی ناوکی بۆ بەکتریای گۆیی هێشویی *Staphylococcus aureus* ی بەرهەڵستکاری Methicillin لە کوردستان–عێراق

پـوخـــتە

لەم توێژینەوەیەدا کۆی گشتی ٢٤١ نمونەی سەرجێیی گرنگ کۆکرانەوە لە سێ نەخۆشخانەی جیاوازی هەرێمی کوردستان کە تیایدا ٦٤ جیاکراوەی بەکتریای گۆیی هێشویی *Staphylococcus aureus* جیاکرانەوە کە دەکاتە ڕێژەی ٢٦,٥٪ی کۆی گشتی نمونە وەرگیراوەکان. ئەنجامەکان پشتڕاست کرانەوە بە بەکارهێنانی تەکنیکی PCR و دەستپێکەریnuc ی تایبەت بە ناسینەوەی ئەم بەکتریایە بە دوهێندکردنی گورزەی ٢٨٠ نیوکلیۆتایدی بۆ بۆهیلی *nuc*. ئەنجامەکان دەریانخست کەوا ڕێژەی جیاکراوەی بەکتریای گۆیی هێشویی بەرگریکاری Methicillin لە کوردستاندا بەرزەو٣١ جیاکراوە بەرگریان پەیداکردبوو بۆ دژەبەکتریای Methicillin کە دەکاتە ڕێژەی (٤٨,٤٪)ی کۆی گشتی جیاکراوەکان. پەیداکردنی بەرگری بۆ Methicillin پشتڕاست کرایەوە بە بەکارهێنانی دەستپێکەری mecA ی تایبەت بە بۆهێڵی *mecA* بە دوهێندکردنی گورزەی ٣١٠ نیوکلیۆتایدی بۆ بۆهیلی *mecA*. سێ جیاکراوەی بەرگریکاری Methicillin کە بریتی بوون لە (SaDu5، SaEr17 وSaSul24) دەستنیسانکران بۆ شیتەڵکردنی زنجیرەی ترشی ناوکی بۆهێڵی *mecA* بۆ هەڵسەنگاندنی پەیوەندی بۆماوەیی نێوان جیاکراوەکانی کوردستان لەگەڵ جیاکراوەی ولاتانی تر لە بانکی بۆهێڵی جیهانی و دۆزینەوەی لێکچونی نێوانیان. ئەنجامەکانی شیتەڵکاری نێوان جیاکراوەکانی کوردستان دەریانخست کەوا ڕێژەی لێکچونی زنجیرەی ترشی ناوکی بۆهیلی *mecA* لە نێوان ٩٧,٥–٩٨,٥٪ بوو. لەکاتێکدا ڕێژەی لێکچونی زنجیرەی ترشی ناوکی بۆهیلی *mecA* لە نێوان جیاکراوەکانی کوردستان و جیاکراوەی ولاتانی تر لە بانکی بۆهێڵی جیهانی لە نێوان ٩٢,٦–٩٦,١٪ بوو. ئەنجامەکانی ریزکاری و بەراوردکاری زنجیرەی ترشی ناوکی ئاشکرایانکرد کەوا لێکچون و چۆنیەکی هەیە لە نێوان جیاکراوەکانی کوردستان و جیاکراوەکانی (Turkey/HM5, UKNTCC124, South Korea/YSSA11 و India/TN/CN/1/12) کە لە داتابەیسی بانکی بۆهێڵی جیهانی دەست دەکەون. بە بەکارهێنانی ئەنجامەکانی ریزکاری و بەراوردکاری و شیتەڵکاری زنجیرەی ترشی ناوکی بۆهیلی *mecA* و لەڕێگەی بەکارهێنانی بەرنامەی کۆمپیوتەری MEGA5 بێتا ڤێرشنی ٦,١ لەسەر ئەنتەرنێت پەیوەندی بۆماوەیی نێوان جیاکراوەکان دۆزرایەوەو پۆلێن کران بۆ چەندین گروپی بۆماوەیی. ئەم توێژینەوەیە بیرکردنەوە دەدات بە دەستەوە لەسەر ناسینەوەی گەردیلەیی بەکتریای *S. aureus* و دۆزینەوەی دژە بەکتریای دروستکراو یان ئەندامی باشتر بۆ کۆنترۆل کردنی دەربڕینی بۆهێڵی بۆ دژە بەکتریاکان لە زیندەوەری توشکەری نەخۆشی جۆراوجۆر بەشێوەیەکی گشتی و بەتایبەتیش بۆ وەچەکانی MRSA.

تحليل تسلسل النكليوتيدي لبكتريا *Staphylococcus aureus* المقاومة ل Methicillin في كوردستان العراق

الخلاصة

في هذه الدراسة تم جمع ٢٤١ عينة سريرية مهمة من ثلاث مستشفيات مختلفة في اقليم كوردستان وتم حصول على ٦٤ عزلة لجرثومة المكورات العنقودية الذهبية *Staphylococcus aureus* التي تمثل ٢٦,٥% من عدد العينات. تم تاكيد الجزيئي للنتائج باستخدام تقنية PCR و بواسطة بادئ nuc المتخصص لجين*nuc* الذي انتج حزمة متضاعفة بوزن جزيئي ٢٨٠ نكليوتيد لجين*nuc*. أظهرت النتائج أن معدل انتشار مقاومة *S.aureus* في كوردستان كانت مرتفعة جدا، حيث وجدت ان ٣١ من هذه العزلات (٤٨,٤٪) كانت مقاومة ل Methicillin. تم تاكيد مقاومة هذه العزلات ل Methicillin باستخدام بادئ mecA المتخصص لجين *mecA* الذي انتج حزمة متضاعفة بوزن جزيئي ٣١٠ نكليوتيد لجين *mecA*. تم اختيار ثلاث عزلات (SaDu5، SaEr17 وSaSul24) المقاومة ل Methicillin لتحليل تسلسل النكليوتيدي لجين *mecA* لتقييم العلاقة بين هذه العزلات والعزلات العالمية المختارة من بنك الجينات و تحديد التشابه بينهم. بينت نتائج التحليل أن التشابه تسلسل جين *mecA* بين العزلات كوردستان تراوحت بين ٩٧,٥-٩٨,٥٪، في حين كان التشابه تسلسل جين *mecA* بين العزلات كوردستان والعزلات العالمية المختارة من بنك الجينات تراوحت بين ٩٢,٦-٩٦,١٪. كشفت نتائج المقارنة بان تسلسل النكليوتيدي لجين *mecA* لعزلات كوردستان وجدت التشابه والتماثل الجزئي مع عزلات (Turkey/HM5, UKNTCC124, South Korea/YSSA11 و India/TN/CN/1/12) المتوفرة في قاعدة البيانات العامة. تم إنتاج شجرة الوراثية من اصطفاف السلسلة المتعددة من تسلسل باستخدام MEGA5 بيتا الإصدار ٦,١ عبر الإنترنت، وأظهر ارتباط نموذجي بين العزلات كوردستان والعزلات العالمية المختارة من بنك الجينات. هذه الدراسة تعطي أفكارا في التشخيص الجزيئي للبكتريا *S. aureus* وللعثور على أفضل المضادات الحيوية الاصطناعية أو العضوية للسيطرة على التعبير الجيني للمضادات الحيوية في الكائنات الحية المسببة للأمراض المختلفة بشكل عام وعلى وجه الخصوص سلالات MRSA.

A COMPARISON OF DIFFERENT METHODS FOR RNA AND DNA EXTRACTION FROM FORMALIN – FIXED PARAFFIN-EMBEDDED TISSUES FROM DIFFERENT CANCER SAMPLES

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Abstract

RNA and DNA extracted from formalin-fixed paraffin-embedded (FFPE) tissue is problematic due to chemical modifications and continued degradation over time. we compared quantity of RNA extracted by two different protocols from 14 recently archived from patients suffered from different cancers distributed among formalin-fixed paraffin-embedded (FFPE) breast cancer tissues ,thyroid cancer tissues and Cervical uterus carcinoma tissues by using Guanidine isothiocyanate (GTC)with phenol-chloroform (protocol-1) and Silica Gel Column(SGC) dependent on spin column purification-based (protocol- 2), to assess, which technique is the most efficient and reproducible in terms of total yield and purity. The results showed RNA isolated with SGC technique was characterized by higher mean concentration in a range (80-180) µg/ml ,but it give positive results to 12 sample with degradation in comparison with RNA isolated by the (GTC) technique (protocol-1), comparison with total RNA extraction from human blood (two distinguished bands) .

In this study comparative methods have been performed to analyze the efficiency for extraction and purification of Genomic DNA from six selective FFPE Tissues samples, revealing that the extraction of DNA by using extraction modified method give good result with yield higher mean concentration of DNA in a range (160-260) µg/ml.

The lysis of FFPE Tissues was enhanced by increased the concentration of proteinase K to30 mg\ml for 2 hour at 65C, which considered the best time for lysis tissues and heated comparable with results that obtained from lysis tissues by using 20mg\ml for (24 -48 hours) at 55C .

Key Words : DNA extraction, RNA extraction, Formalin – Fixed Paraffin-Embedded Tissues (FFPE).

Introduction

The identification and validation of molecular markers in formalin-fixed, paraffin-embedded (FFPE) tissue is currently an area of intense and exciting research activity. This is due in part to the fact that expression profiling, genotyping, and mutation analysis have been shown to aid in diagnosis and to provide guidance, particularly in the treatment of cancer. Despite this concentrated effort, to date, only a limited number of individual markers or panels of markers using extracted nucleic acids from histopathological tissue specimens have been introduced into routine clinical practice (Hennig, *et al.*,2010)..

Formalin-fixed, paraffin-embedded (FFPE) tissue is one of the most widely practiced methods for clinical sample preservation and archiving. It is estimated that, worldwide, over a billion tissue samples, most of them FFPE, are being stored in numerous hospitals, tissue banks, and research laboratories. FFPE samples pose a major challenge for molecular pathologists, because nucleic acids are heavily modified and trapped by extensive protein-nucleic acid and protein-protein cross linking. Historically, Recently, however, it has been discovered that with appropriate protease digestion, it is possible to release microgram amounts of DNA and RNA from FFPE samples. The purified nucleic acids, although highly fragmented, are suitable for a variety of downstream genomic and gene expression analyses, such as polymerase chain reaction (PCR), quantitative reverse transcription PCR (qRT-PCR), microarray, array comparative genomic hybridization (CGH), microRNA, and methylation profiling. Several commercial kits are currently available for FFPE extraction (Weining *et al*.,2009)..

The recovery of nucleic acids (DNA and RNA) from fixed, paraffin-embedded specimens is challenging. Although formaldehyde (HCHO), a principal ingredient of most commonly used fixatives, does not physically degrade nucleic acids, it leads to the generation of DNA-protein and RNA-protein. crosslinkages. Furthermore, the nucleic acids will fragment in situations where the fixative solution is unbuffered, as the pH can be extremely low (Thomas *et al*.,2007). Furthermore, many methods for extracting nucleic acid from FFPE tissue, particularly those that are highly manual, are not standardized protocols , In addition, these manual protocols

are time consuming and did not require to use of hazardous and flammable materials like xylene and ethanol for deparaffinization. The GTC technique for isolation of RNA, is very popular because it requires much less time than other classical methods. Moreover, GTC salt denatures the cellular proteins and inactivates RNases ensuring that isolated RNA is not degraded and separates rRNA from ribosomes,. Such factors have required individualized adaptation to create standardized protocols. As a result, these isolation protocols have been incompatible with high-throughput formats (Benavides.*et al*.,2006). Several attempts have been made to isolate RNA from formalin-fixed paraffin-embedded tissue using modifications of currently available techniques for RNA extraction . However, the interpretation of results is often difficult and therefore, alternative methods for long-term storage of tissues prior to gene expression profiling are greatly required (**Muyal *et al*.,2009).** So the present study was designed to test the efficiency of RNA and DNA extraction from archival formalin fixed, paraffin-embedded tissues based on quantity of the nucleic acid extracted: The goals included development of a simpler and more effective protocol for RNA and DNA extraction .

MATERIALS AND METHODS

Formalin-fixed paraffin- embedd (FFPE)tissue blocks

This study included 14 FFPE blocks of different cancer tissues from different ages (those with better fixation& processing) collected from the department of Histopathology –Teaching Laboratories that belongs to the Medical City Teaching Hospital during the period of study from November 2011 to February 2012., by archival paraffin-embedded tissue blocks along with the histopathological reports for 14 patients with tumors patients` ages and tumors stage were . These samples were distributed as follows: Breast cancer (6), Thyroid cancer (6) , Cervical uterus carcinoma (2), in different ages (25-65).

Sectioning the paraffin –embedded blocks.

For each paraffin block, one 10 micrometer thick section was cut using rotary microtome (Leica, Germany) (more than 5 section for each sample) and collected in each sterile eppendorf tube, ensuring that an equivalent amount of tissue was placed in all the eppendorf tubes(Thomas *et al*, 2007).

Deparafinization method

Deparafinization was carried out by adding 1ml of xylene to each tube containing the tissue sections, and this was vigorously vortexed and Incubated at 55 C 20 minutes. Centrifugation was then performed at full speed for 5 minutes, and the resulting supernatant was discarded. The deparaffinization step was repeated once again, followed by the addition of 500ML of absolute ethanol, and this was mixed by vortexing for 1 minute .Incubated at 55 for 15 min The solution was then centrifuged at full speed for 5 minutes, and the resulting supernatant was discarded ,This step was repeated once again,.

Extracted RNA From tissue after Deparafinization (protochol-1)

After the steps of Deparafinization of the sections, to extracted RNA from tissue, the pellet was resuspended in 500 µl of Lysis buffer (SDS 2%, Na2-EDTA 1mM, Tris – HCl ,20mM , Guanidine isothiocyanate (GTC)1M, Mercabto ethanol , 25mM , DEBC (ddH2O), and homogenized using vortex for minutes , then Add 300 µl of a freshly prepared solution of Proteinase K (6 mg /ml) and incubated at 55ºC for (2- 24) hours. After lysis cells and extracted RNA from tissue we continuous other steps for purified RNA by addition 500 µl phenol : choloform: isopropanol alcohol at 25:24:1, followed by vigorous vortexing and centrifugation at 12,000 x g at room temperature for 10 minutes. The solution at the aqueous phase was transferred to a new 1.5 ml microfuge tube and an equal volume of chloroform was added, followed by mixing by vortexing and centrifugation at 12,000 x g for 5 minutes. RNA precipitation was performed by the addition of 0.1 volume of 3 mol/L sodium acetate (pH 4.0), an equal volume of isopropanol and 1 µl of 10 mg/ml carrier glycogen, followed by incubation overnight at – 20 C. The mixture was centrifuged at 12,000 x g at 4 C for 5 minutes. The supernatant was discarded, followed by washing of the RNA pellet with 500 µl of 70% ethanol and air-dried aseptically. The air-dried RNA pellet was resuspended with 30 µl of RNase-free water (Specht *et al*., 2001) .

Extracted RNA From tissue after deparafinaization (protochol-2)

After the steps of Deparafinization of the sections, RNA extracted from tissue , The pellet was resuspended in 500 µl of Lysis buffer (SDS 2%, Na2-EDTA 1mM, Tris – HCl ,20mM , Guanidine isothiocyanate (GTC)1M, Mercabto ethanol , 25mM , DEBC (ddH2O), and

homogenized using vortex for minutes , then Add 300 µl of a freshly prepared solution of Proteinase K (6 mg /ml) and incubated at 55ºC for 2 hours only. After lysis cell and extracted RNA from tissue we continuous other steps for purified RNA according to the Total RNA Mini Kit instead of manual method(protochol-1) for purification as described in a manual of mini kit by using silica column (Rupp and Locker 2001).

Extracted RNA From Blood

In this study ,Total RNA Mini Kit (Blood Cultured Cell protocol , promega \USA) was used to extract RNA from blood (normal person as a standard).

Extracted DNA from tissue after deparafinaization

In this study the method described by Thomas *et al* (2007) was used with some modification to isolate genomic DNA from 3 FFPE samples of breast cancer type and 3 FFPE samples of thyroid cancer type .After the steps of Deparaffinization of the sections ,Adding 300µl of Proteinase K buffer in final concentration 20mg/ml to the extracted sample with lysis buffer 1 .Incubation at 55°C for 24 hours, to assure the sample lysate is clear, during incubation the sample should be inverted every 30min (We repeated this step when the sample lysate is not clear by adding 300µl of Proteinase K buffer in final concentration 20mg/ml to the sample extraction and Incubated 24 hours at 55°C until the sample lysate become clear) . Adding 500µl of chloroform: isoamyl alcohol in percentage(24:1) to extract and mixing by shaking vigorously for 30 min until the mixture emulsified, and then centrifuged at 12000 rpm for 20 minutes.The aqueous phase was transferred to another tube (avoiding touching the interphase layer), two volumes of cold absolute ethanol was added to the aqueous phase and immediately mix by shaking vigorously for 10 seconds. and then were kept at –20 ºC overnight. The samples were centrifuged at 12000 rpm for 20 minutes, the supernatant was discarded and the precipitate was left to dry completely. Adding 500µl of 70% ethanol. and discarding the flow-through. The precipitate was dissolved in 50 µl of TE buffer .The samples were then kept at –20 ºC.

Extraction treatments: the effect of cell lysis buffers

To investigate the effect of cell lysis buffers on nucleic acid quality, samples were compared across the following buffers: cell lysis 1(SDS 10%, Na2-EDTA 0.5M, Tris – base 1M) (Shi *et al* , 2002), cell lysis2(SDS 0.5%, Na2-EDTA 0.1mM, Tris – base 10mM)(Bohmann *et al*., 2009) and cell lysis 3(SDS 2.0%, Na2-EDTA 2.5mM, Tris – base 25mM ,Sodium citrare 25mM, CaCl2 5mM (Thomas *et al*., 2007).

Extraction treatments: the effect of incubation time Lysis buffer and temperature .

To investigate the effect of incubation time in nucleic acids extraction, pairs of samples were tested across the following incubation times: 2, 24 hours. After we determined the best cell lysis buffer, the samples were tested across the incubation times 2 hours at 65 ºC.

DNA Extraction from Human blood

This method described by Sambrook and Rusell (2001) to isolate genomic DNA from human blood (used as control) ,Approximately 3-5 ml of blood was taken from donor by sterile syringe and places in EDTA tubes..

Agarose Gel Electrophoresis

Agarose gel was prepared according to the method described by Sambrook and Rusell (2001). Agarose gels were stained with ethidium bromide (0.5µg/ml) for 30 minutes. DNA bands were visualized under U.V transilluminator at 365 nm wavelength. A gel documentation system was used to document the observed bands.

Estimation of the nucleic acid concentration by the Spectrophtometer

The purity and concentration of the extracted DNA and RNA were determined by UV-Spectrophotometer (Eppendorf, Hamburg, Germany), according to the Protocol as described by Sambrook and Rusell (2001).

Results and Discussion

Isolation of Nucleic acid

The Comparison of Two Techniques for Total RNA Isolation from FFET Samples

In this study comparative methods have been performed to analyze the efficiency for extraction and purification of total RNA from 14 samples of FFET from different cancer types, revealing that the extraction RNA according to protocol 1 suffer from low efficiency, mainly due to incubated the samples over night and purified by phenol-chloroform this lead to degraded [figure(1),lane 11, lane12 , lane13 (present in small amount), in compare with

extracted RNA according to protocol 2 by using Guanidine Isothiocyanate (GTC) and Silica Gel Column(SGC) techniques (spin column purification-based protocols) , although the protocol 2 give positive results to 12 sample with degradation as showed in (figure -2) comparison with total RNA extraction from human blood (figure (1) lane2, lane3). While we did not get any result from samples of cervical uterus carcinoma by using two protocol (data not shown).

As we know about Formalin fixation, the most widely used fixative in histopathology, has many advantages such as the ease of tissue handling, the possibility of long-term storage, an optimal histological quality and its availability in large quantities at low price. But RNA is a particularly labile bio-molecule and is much more susceptible to degradation by endogenous- and exogenous-nucleases and to non-specific degradation by divalent cations, heat, elevations in pH, and storage of tissue or cells over extended periods prior to RNA extractions, which result in falsely altered gene expression patterns. However, this technique poses many problems due to the fact that formalin fixation cross-links nucleic acids and proteins. Further, mono-methylol is added to the amino groups for all four RNA bases ($N\text{-}CH_2OH$) and subsequently methylene bridges are formed between neighbouring bases that resulted to continue degradation over-time (Masuda *et al* .,1999). or the extremely low pH (<1). Of the fixative.(Gillio *et al*.,2007). The two most reliable and widely used techniques for high throughput RNA isolation are: 1- Guanidine Isothio Cyanate-phenol:chloroform (GTC)-based RNA isolation technology and , 2- Silica-gel column (SGC)-based RNA isolation technology. In contrast, the principle of SGC technology is a combination of the selective binding properties of a silica-based membrane with the speed of microspin technology, which allows saving time, money, and efficient use of small and precious biological samples.

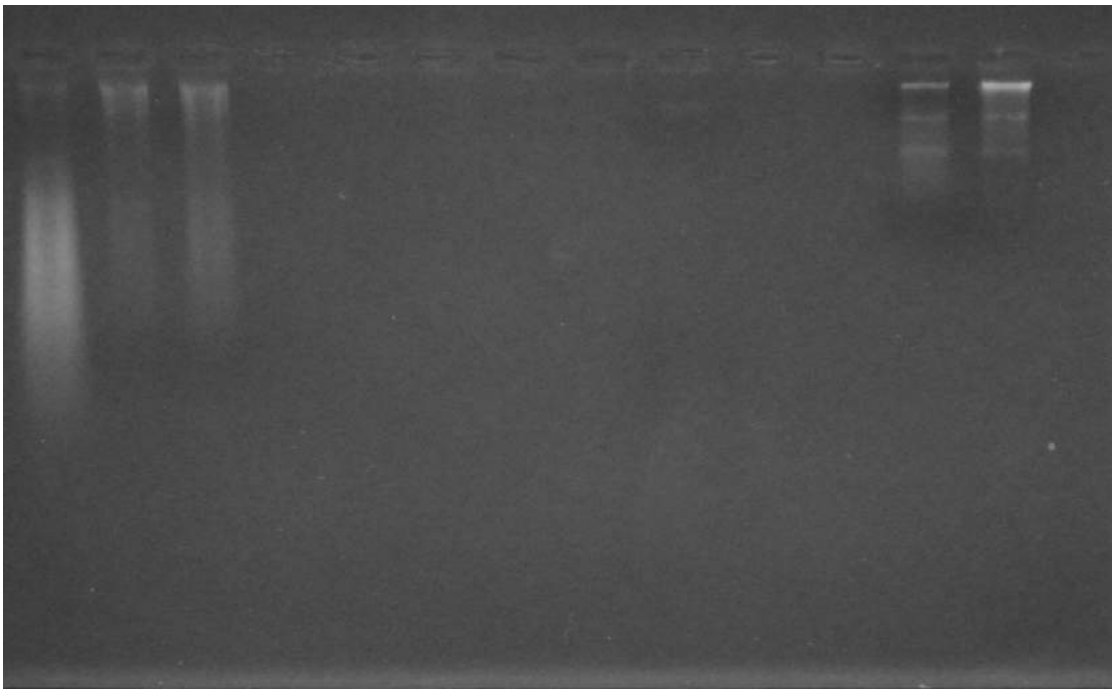

Figure (1) : Agarose gel electrophoresis of RNA Extraction from FFPE Tissues from different cancer types By using protocol 1, with agarose concentration (1.5%),voltage 5volt/cm,during 2 hr

Lane 2 and 3 : RNA extract from human blood (control).
Lane 1,4,5,6,7,8, : RNA extract from Thyroid cancer type.
Lane 9,10,11,12,13,14 : RNA extract from breast cancer type

Here in this study , compare RNA extraction protocols on a set of 14 FFPE different cancer samples, testing spin column purification and phenol-chloroform technologies, That we designate Unfortunately formalin fixation induces RNA-tissue protein cross-links, which can prevent obtaining a good results or degraded RNA In addition, nucleic acid fragmentation may occur in formalin fixed tissue due to aging of the specimen or the extremely low pH (<1). of the fixative.

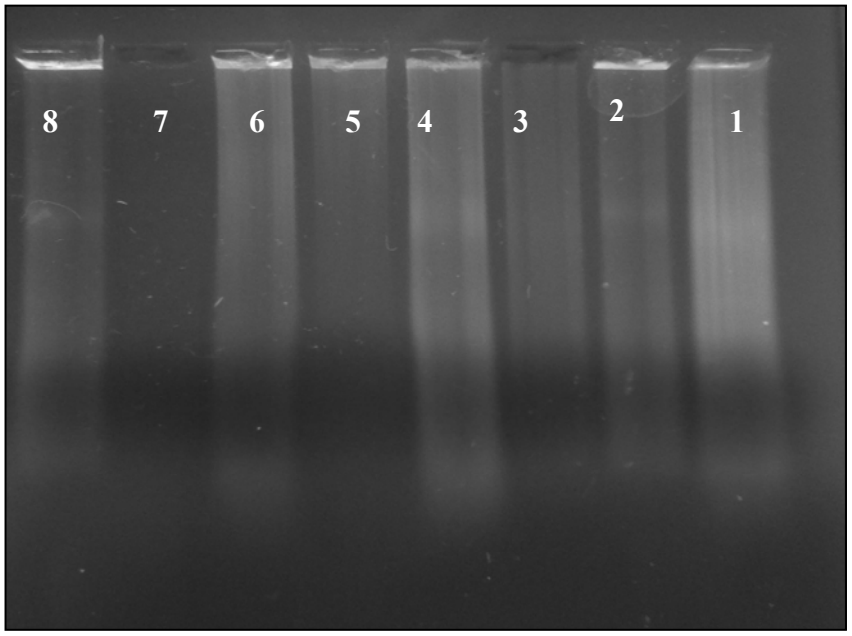

Figure (2) : Agarose gel electrophoresis of RNA Extraction from FFPE Tissues from different cancer types By using protocol 2, with agarose concentration (1.5%),voltage 5volt/cm,during 2 hr

Lane 1 ,2,3,4 : RNA extract from Breast cancer type.
Lane,5,6,7,8, : RNA extract from Thyroid cancer type.

The Modified Extraction methods To extract DNA from FFPE Tissues samples

This study shows how the modified extracted method can actually give good yield of DNA. Three different lysis digestion buffers of DNA extraction were tested with six FFET samples , and the results showed the best lysis buffer extract was type (3) (figure -5) in DNA concentration range (200-260) **µg/ml** , compare with other results as showed in (figure - 3) and (figure - 4). In figure (6) three sharp band appeared in three samples of FFPE Tissues, this results revealed to the modified DNA extraction method (the incubation time 2 hours , the temperature of lysate cell 65 C , the concentration of proteinase K 30mg\ml and add GTC(1M) to the best digestion buffer).In other words, thermal energy does not lead to an increase of extracted nucleic acids; it merely makes whatever available DNA and RNA more amenable to amplification, presumably by making it less cross-linked (Banerjee *et al* .,1995).

So the data (only three samples) shows no indication that even 24 hour or 48 hour digestions at 55C adversely effect the DNA yields with increased incubation temperatures, this may become an important factor However, comparisons between digestions at 65C and higher temperatures provide evidence , the higher temperatures significant DNA and RNA degradation occurs (85C versus 65C, up to 8000 times less PCR amplifiable, average/standard deviation Therefore, there is good evidence to argue that increased digestion temperature can be useful with regard to obtaining greater levels of PCR amplifiable DNA; however, these digestion temperatures should be limited to 65C(Shi *et al* .,2002).

In this study , lysis of FFPE Tissues was enhanced by increased the concentration of proteinase K to30 mg\ml for 2hr at 65C , which considered the best time for lysis tissues and heated comparable with results that obtained from lysis tissues for 24 -48 hours. The data presented here indicate that proteinase K digestion is required for obtaining DNA of sufficient quality by all 4 extraction methods. DNA depended on the extraction method. On types of lysis buffers extraction and heat-

treatment in combination with proteinase K digestion resulted in the good quantity of DNA concentration were found in four extracts of each samples after proteinase. K digestion and heat-treatment. This may in part explain why it is not unusual to find conflicting findings in previously published studies. Such as, in a comparison of the effect of time of incubation during tissue digestion, Isola *et al.* (1994) argue that prolonged time is better, while Banerjee *et al.* (1995) argue that no more than 3 hours are required. While others study pointed to importance of adding Proteinase K in high concentration and incubation at high temperature (60 to 70°C) in DNA extraction method (Thomas *et al*., 2007) ,this also lead to removes part of the methylol additions induced by formalin fixation . The predominant alternative to Tris-based digestion buffers are guanidinium thiocyanate/proteinase k containing buffers, favored by those who perform DNA extractions using commercially available kits (e.g. Qiagen's QIAamp DNA micro kit) that are based on the silica-binding principle described by Boom et al. (1990). In this study we succeeded in finding a modified method for DNA isolation especially that from FFPE Tissues samples by decreased the incubation time to 2 hours and increased the temperature of lysate cell to 65 C with increase the concentration of proteinase K to 30mg\ml with addition the GTC(1M) to the best digestion buffer .

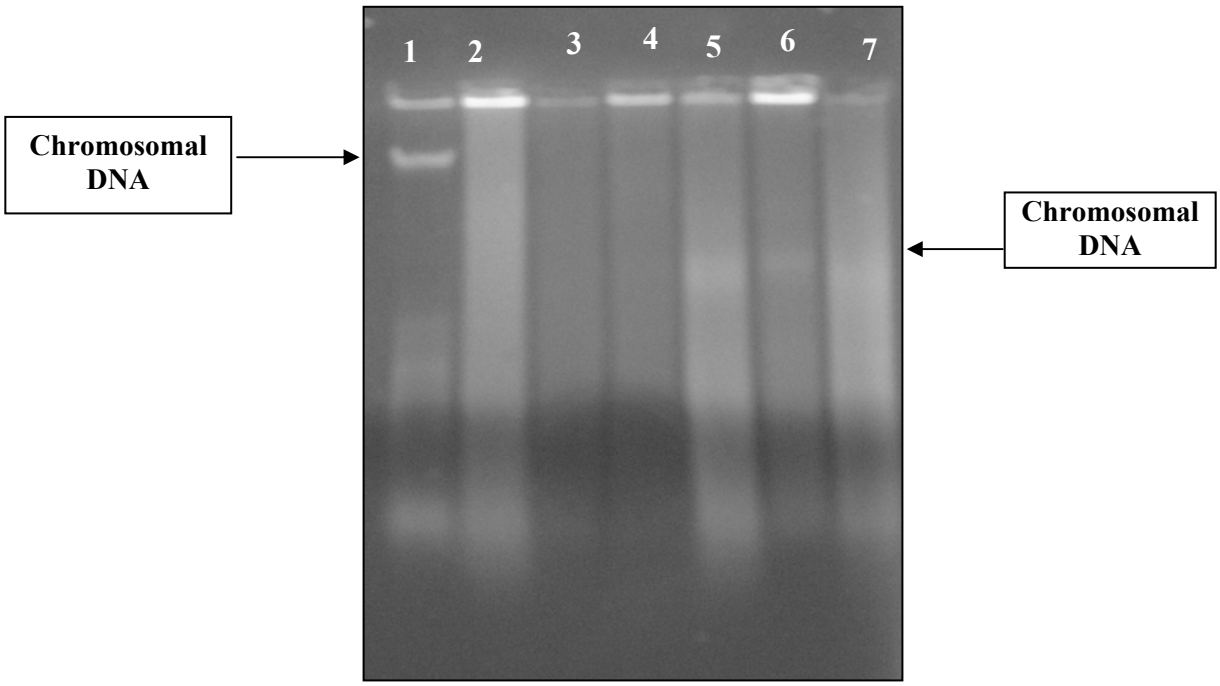

Figure (3): Effect of cell lysis buffer type(1) on detection the quantity of DNA extraction from different samples of cancer by agarose gel electrophoresis (with agarose concentration (0.8%),voltage 5volt/cm,during 1.5hr)
Lane 1 : DNA extract from human blood (control).
Lane 2,3,4, : DNA extract from breast cancer type.
Lane 5,6,7, : DNA extract from thyroid cancer type

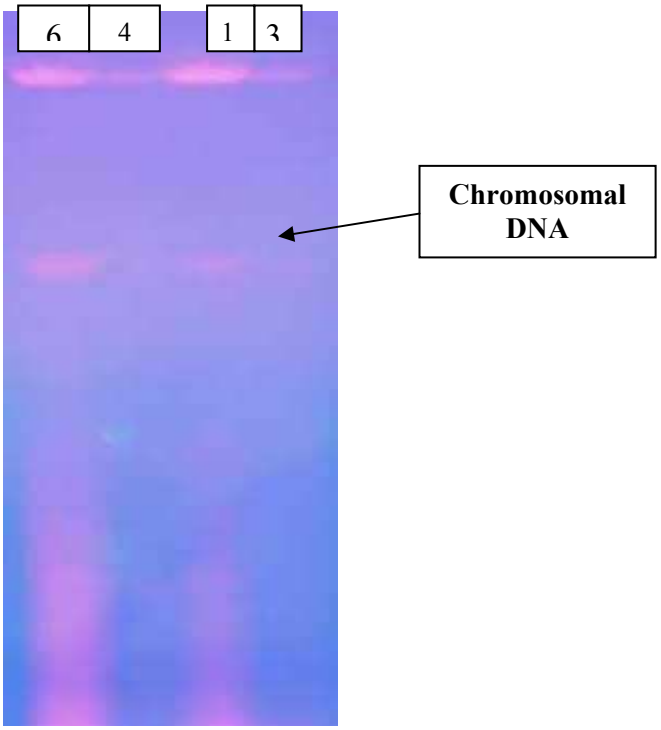

Figure (4): Effect of cell lysis buffer type(2) on detection the quantity of DNA extraction from different samples of cancer by agarose gel electrophoresis (with agarose concentration (0.8%),voltage 5volt/cm,during 1.5hr .

Lane 1 , 3 : DNA extract from breast cancer type.(B)
Lane 4, 6 : DNA extract from thyroid cancer type.(B)

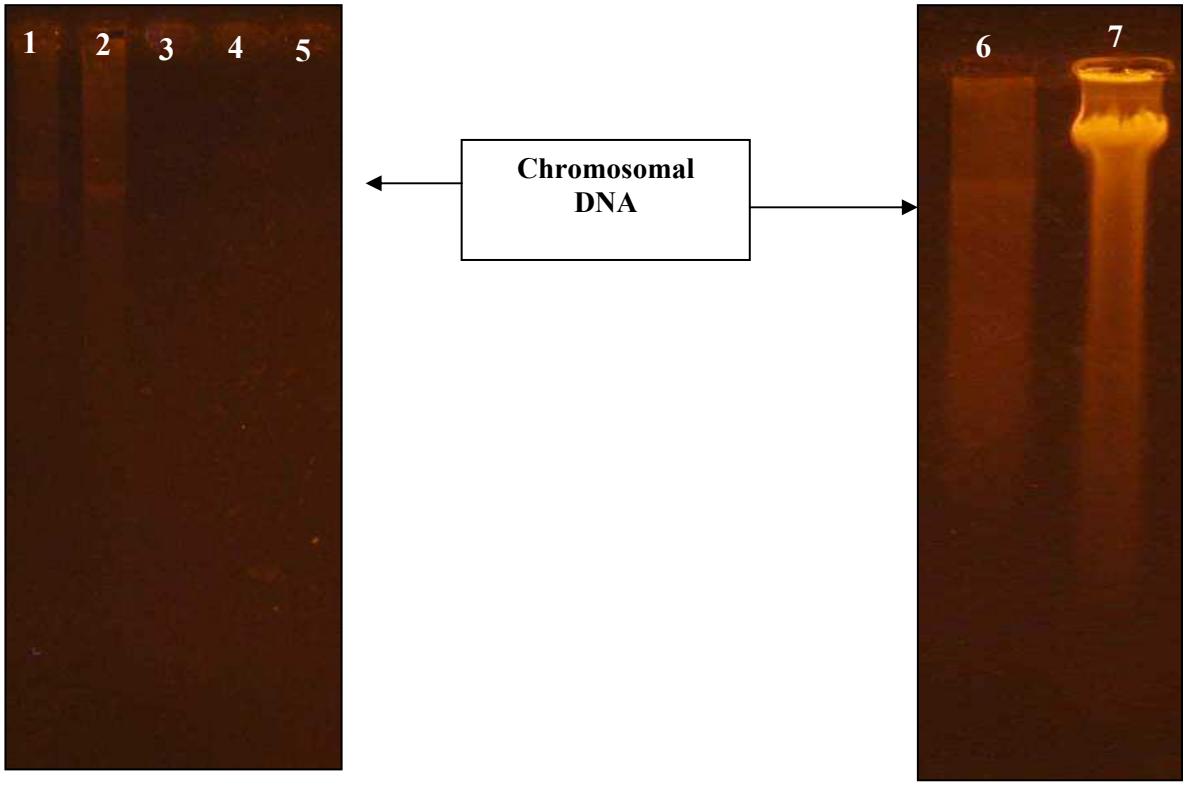

Figure (5): Effect of cell lysis buffer type (3) on detection the quantity of DNA extraction from different samples of cancer by agarose gel electrophoresis (with agarose concentration (0.8%),voltage 5volt/cm,during 1.5hr).
Lane 1 ,2,3, : DNA extract from breast cancer type.
Lane 4,5,6, : DNA extract from thyroid cancer type.
Lane,7 :DNA extract from Human blood sample

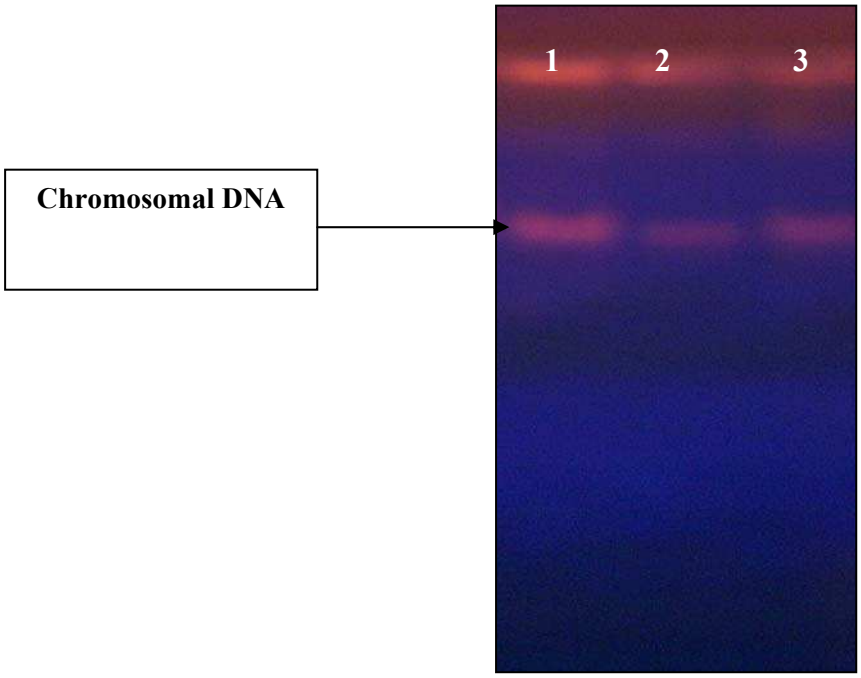

Figure (6): Agarose gel electrophoresis of DNA extracted by using modified methods from different samples of cancer by agarose gel electrophoresis (with agarose concentration (1%),voltage 5volt/cm,during 1.5hr)
Lane 1, 2 : DNA extract from breast cancer type.
Lane, 3 : DNA extract from thyroid cancer type.

Assessment of total RNA yield and purity

The amount of total RNA extracted for each samples was measured by a UV and Vis-Spectrophotometer with the exception of the samples that contained no RNA. According to the results of methods extracted total RNA as mentioned .

The results of protocol (1) showed only three breast cancer samples give result, while tweleve samples from breast cancer and thyroid cancer showed positive result according to protocol 2 .The estimated mean total RNA extracted for protocol (2) was 130 µg (2.6 µg\ µl)(2600 ng/µL) for breast cancer samples with values ranging from 80 to 180 µg (1.6 to 3.6 µg\ µl)(1600 to 3200 ng/µL) , for thyroid cancer samples the estimate mean total RNA extract was125 µg (2.5 µg\ µl)(2500 ng/µL) with values ranging from 70 to 180 µg (1.4 to 3.6 µg\ µl)(1400 to 3600 ng/µL), . The meanA260/A280 ratio for protocol 2 was 1.8 ± 0.2 , The desired ratio A260/A280 is in the range of 1.7 to 2.1 and is dependent on the extraction conditions (Table-1).

The purity of the extracted RNA was comparable throughout the samples and was close to a ratio (A_{260}/A_{280}) of 1.85, the GTC and the SGC technique,. A ratio close to 1.8 indicates that there were only limited protein contamination.

Table (1): Comparative data of total RNA Extracted from different cancer type by modified protocol2,

Types of cancer	A260/A280 ratio.	Yield : µg/ml
Breast cancer (6)	2, 1.25,1.4,2,1.7,1.7	180,80,100,160,110,120,
Thyroid cancer(6)	1.7,1.3,2,1.7,1.8,2.3,	120,70,150,120,123,180
Cervical uterus carcinoma	-	-

* The total yield, 1.8 < A260/A280 ≥ 2 indicates pure RNA

Assssement of DNA yield and purity

According to the results of methods extracted DNA as mentioned above . The results of Modified methods by using different lysis buffers, showed only three breast cancer samples give positive result, while others samples from thyroid cancer did not show any result . To evaluate the purity of the extracted DNA, absorbance ratio at 260/280 nm (DNA/protein) was determined. (Table 2). The estimated mean total DNA extracted by using digestion lysis buffers type(1) was 170 µg (3.4 µg\ µl)(3400 ng/µL) for breast cancer samples with values ranging 160-180 µg (3.2-3.6 µg\ µl)(3200 -3600 ng/µL) , for digestion lysis buffers type(2) the estimate mean total DNA extract was200 µg (4 µg\ µl)(4000 ng/µL) with values ranging from 160 to 240 µg (3.2 to 4.8 µg\ µl)(3200 to 4800 ng/µL), for digestion lysis buffers type(3) the estimate mean total DNA extract was230 µg (4.6 µg\ µl)(4600 ng/µL) with values ranging from 200 to 260 µg (4 to 5.2 µg\ µl)(4000 to 5200 ng/µL), while the value for modified method the estimate mean total DNA extract was250 µg (5 µg\ µl)(5000 ng/µL) with values ranging from 220 to 280 µg (4.4 to 5.6 µg\ µl)(4400 to 5600 ng/µL),.. The meanA260/A280 ratio for Extract DNA with different lysis buffers was 1.6 and for modified method 1.8 – 2 , The desired ratio A260/A280 is in the range of 1.6 to 2 and is dependent on the extraction conditions .

Table (2): Comparative data of total DNA Extracted from different samples of cancer by modified protocol,

Types of cancer	A260/A280 ratio.	Yield : µg/ml
Breast cancer (1)	2* , 1.7**, 2***	180, 160, 210
Breast cancer (2)	1.7 * , 0 ** , 1.8***	160, 0 , 200
Breast cancer (3)	1.6* , 0** , 0 ***	160 , 0 , 0
Thyroid cancer (4)	-	
Thyroid cancer (5)	-	
Thyroid cancer (6)	0 *, 1,8** , 1,9***	0 , 240 , 260
	Modified method	
Breast cancer (1)	1.88	260
Breast cancer (2)	1,8	220
Thyroid cancer (6)	2	280

*** The total yield, 1.8 < A260/A280 ≥ 2 indicates pure RNA**

* result of digestion lysis buffer (1) ** result of digestion lysis buffer (2) *** result of digestion lysis buffer (3).

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بەکارهێنانی ڕێگەی جیاواز بۆ جیاکردنەوەی ناوکە ترشی DNA وRNA لە شانەی چاندێندراو لە ناو مۆمدا لە سامپڵە شێرپەنجە جیاوازەکاندا

پوختە

چەند کێشەیەك هەیە لە ڕێگاکانی جیاکردنەوەی DNA وRNA لە شانە چاندێندراوەکانی ناو مۆم ئەمەش بەهۆی گۆرانکاری کیمیای و ئەو پارچانەوەی کە دروست بوون بە تێپەڕبوونی کات ، ئەم توێژینەوەیە پێکهاتبوو لە ڕێگا چەندێتیەکان لە جیاکردنەوەی RNAلە ١٤ سامپڵی شێرپەنجەیی چاندێندراوهلە مۆمدا بۆ ماوەی (٢-٥ مانگ) لە چەند نەخۆشێکی شێرپەنجەیی جیاواز وەرگیرابوون وەکو شێرپەنجەیی ممەك و گلاندنی دەرەقی و شێرپەنجی ملی منداڵدان ، بە بەکارهێنانی دوو ڕێگا جێ بەجێ کرا یەکەمیان بە بەکارهێنانی Guanidine isothiocyanate (GTC)لەگەڵphenol-chloroform وە ڕێگەی دووەم بە بەکارهێنانی(Silica Gel Column(SGC بۆ دیاریکردنی باشترین ڕێگا بۆ پوختە کردن و بەتوانیی پوختێتی وە چڕی RNA. لە دەرەنجامدا ڕێگەی دووەم بە تواناتر بوو SGC لە پوختەکردنی RNAلە هەر ١٢ سامپڵە کەی شێرپەنجەی مەمك و گلاندی دەرەقی و بە چڕی (٨٠-١٨٠)مایکرۆگرام / ملبە بەراوردکردنی لەگەڵ ڕێگەی یەکەمدا کە(GTC)کە تەنها لە٣ سامپڵی شێرپەنجەیی مەمکی بەکارهێنا بۆ جیاکردنەوەی RNAبە بەراورد کردنی لە RNA جیاکراوەکان لە خوێنی ئادەمیزاد (دەرکەوتنی دوو گورزە).

هەروەها ئەم توێژینەوەیە چەند شتێکی تریش ئەگرێتەوە وەك دۆزینەوەی ڕێگەی باش و بە توانا بۆ تەکنیکی جیاکردنەوە DNA بۆ ٦ سامپڵی هەڵبژێردراو لە شانە چێندراوەکانی ناو مۆم، ئەنجام بە توانای ڕێگایی پوختە کردنەکە گۆراوەکەی دەرخست بە بەکارهێنانی ٣ گیراوەی جیاواز بۆ تێکشکاندنی خانەکان کاتێك کە چڕیەکی DNA ی کرد بە (١٦٠-٢٦٠) مایکروگرام/ مل ، وەباشترین چڕی بۆ ئەنزیمی شیکەرەوەی شانە ٣٠ مل گرام / مل لەکاتی ماوەی هەڵگرتن ١٢٠ خولەك و پلەی گەرمی ٦٥ سەدی بە بەراورد کردنی لەگەڵ ئەو ئەنجامانەی کە دەست نەکەوت لە شیکردنەوەی شانەکانبە چڕی ٢٠ مللگرام / مل بۆ ماوەی (٢٤-٤٨) کاتژمێر لە پلەی گەرمی ٥٥ سەدیوە چڕیDNA (٢٢٠-٢٨٠)مایکروگرام /مل.

استخدام طرق مختلفة لاستخلاص الـ RNA و DNAمن الانسجة المطمورة في الشمع من عينات سرطانية مختلفة

الخلاصة

تعاني طرق استخلاص RNA و DNA من الانسجة المطمورة في الشمع من مشاكل عديدة ويعود هذا الى التحويرات الكيميائية والقطع الناجمة بمرور الزمن , تضمنت الدراسة مقارنة بين الطرق الكمية في استخلاص الرنا من ١٤ عينة سرطان مطمورة في الشمع جمعت من مرضى يعانون من امرا ض سرطانية مختلفة توزعت مابين عينات سرطان الثدي وسرطان الغدة الدرقية وعنق الرحم، باستخدام طريقتين تمثلت الاولى باستخدام (GTC) Guanidine isothiocyanate مع phenol–chloroform والطريقة الثانية باستخدام (SGC)Silica Gel Column لتحديد الطريقة الكفوءة في الاستخلاص ودرجة النقا وة وتركيز الرنا . واظهرت النتائج كفاءة الطريقة الثانية SGC في استخلاص الرنا من ١٢ عينة لسرطان الثدي وسرطان الغدة الدرقية بتركيز (٨٠–١٨٠) مايكروغرام \مل مقارنة بالطريقة الاولى باستخدام (GTC) اذ اسخلص الرنا من ٣ عينات من سرطان الثدي فقط مقارنة مع الرنا المستخلص من عينة دم بشرية (ظهور حزمتين).

شملت الدراسة ايضاالتحري عن طرق كفوءة في استخلاص الـ DNA وتنقيته من ستة عينات منتخبة من انسجة مطمورة في الشمع، واظهرت النتائج كفاءة طريقة استخلاص محورة باستخدام ثلاث محاليل مختلفة لتكسير الخلايا عندما اعطت تركيز للدنا تراوح (١٦٠–٢٦٠) مايكروغرام \ مل ، وافضل تركيز للانزيم الحال للنسيج ٣٠ مللغرام \ مل عند فترة حضانة ١٢٠ دقيقة بدرجة حرارة ٦٥ مئوي مقارنة مع النتائج التي حصل عليها من تحلل الانسجة بتركيز ٢٠ مللغرام \ مل للمدة(٢٤–٤٨) ساعة بدرجة حرارة ٥٥م .

STUDY OF SOME RISK FACTORS IN PATIENTS WITH UNTREATED BREAST CANCER IN ERBIL CITY

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Summary

This study was carried out in Rizgary and Hawler Teaching hospital in Erbil city, from the period of August 2010 to June 2011. A total of 115 women patients with breast tumor (55 malignant and 60 benign) and 40 females as a control group with age ranged (18-71) in both patients and control group were participated, they were matched with patients by age group without any history of breast problem or neoplastic disease and not had any other cancer. Data were collected by interview with patients and control group, a questioner form were provided to each patients which include (age, age at menarche, regularity of menstruation, etc.) and clinical file made for each patient who attends to these two hospitals for each patients for the first time, in spite of histopathological diagnosis. Results indicated obvious relation between risk factor and breast cancer (BC), among these factors is the age; the most frequent age for breast cancer was that at age group (38- 47).Percentage of patients starting age menarche after 12 year was higher when compared with patients starting at or before 12 year. The results showed higher percentage of BC in patients who have regularity menses when compared with irregular menstrual cycle. Age of first pregnancy in 20 years and greater which was higher than other group. The results indicated that the percentage of BBD patients which diagnosed premenopausal was higher than postmenopausal. Higher percentage of breast feeding found in BC patients when compared with bottle feeding. Oral contraceptive pills users occupied higher percentage in patients. Patients which not used hormone replacement therapy (HRT) have higher percentage when compared with patients used HRT. About half of the patients recorded positive family history. The numbers of patients diagnosed as stage third and fourth were higher compared with those stage two, unknown and first stage. According to the type of benign breast disease, the unknown cause showed higher percentage when compared with other types.

Keywords: *Breast cancer, risk factors, epidemiological study.*

This study is a part of M.Sc. Thesis.

Introduction

Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries (World health organization, 2008). It is the most frequently diagnosed cancer and the leading cause of cancer death in females worldwide according to global statistics (2008), accounting for 1.38 million of the total new cancer cases and 458,400 of the total cancer deaths annually (Jemal *et al*., 2011). Breast cancer is the second most common type of cancer after lung cancer. One woman in ten will develop the disease and one in 29 will die as a direct result of it (Wernberg *et al*., 2009). Generally, breast cancer refers to cancer originating from breast tissue (Martini *et al*., 2006). In Iraq, breast cancer is the commonest type of female malignancy, accounting for approximately one- third of the registered female cancers according to the latest Iraqi Cancer Registry (Iraqi Cancer Registry, 2007). The incidence of breast cancer in Erbil city was more common than the other type of cancers (Iraqi Cancer Registry, 2005).

Aim of the study

The present study was aimed to shed light on the risk factors which may use for detection and they are related with breast cancer.

Materials and methods

The study was include 115 female patients presented with breast mass (55 malignant and 60 benign) attended Rizgary and Hawler teaching hospital in Erbil city from August 2010 to June 2011. The age of the patients ranged from 18-71 years and the mean was 44.5 years. A total of forty women selected as control group, they were matched with patients by age group without any history of breast problem or neoplastic disease and not had any other cancer. Data were collected by interview with patients and control group, a questioner form were provided to each patients which include (age, age at menarche, regularity of menstruation, etc.) and clinical file made for each patient who attends to these two hospitals for each patients

for the first time, in spite of histopathological diagnosis.

Results

Age groups of the patients

The highest number of breast cancer patients were found at age interval (38- 47) year, while the lower or minimum record was at aged 58 and older. The higher number about benign breast disease patients was found at the table age interval (28- 37), as well as the minimum number was found in the last age interval.

Patients and age at menarche

In fig: 2 about 45.46% of the breast cancer patients have got their menarche at or before 12, while 54.54% of them got it after 12 years old. However, about the BBD the data is different, 16.67% got their menarche at or before 12 years, and 83.33% got it after 12 years old. Women with early age at menarche or late menopause have an increased risk of developing BC.

Regularity of menstruation and patients

In this study, regularity of menses is considered as a risk factor for BC as showed in (Fig. 3). This study showed 74.54% of BC patients had regular menses, while only 25.46% of them had irregular menstrual cycle. The result for BBD patients showed that 76.66% had regular and 23.34% had irregular menstrual cycle.

Age at first pregnancy of the patients

In fig: 4 about 16.36% and 16.66% of breast cancer and BBD patients were single or infertile, 27.27% of breast cancer patients had first pregnancy before 20 years old, while 56.36% were at 20 or older. However, 36.66% of the BBD patients had first pregnancy before 20 and 46.66% had it at or after 20. About 55% of our patients had their first pregnancy at age 20 or older.

Patients and age at diagnosis

Menopausal age used as a point for this risk factor. 65.46% of the breast cancer patients was diagnosed premenopausal, while it was higher in BBD patients which were 80%. Therefore, 34.54% of the BC patients were diagnosed postmenopausal, whereas for BBD patients were 20% as shown in (fig. 5).

Relation between BC and oral contraceptive pills

In this study, using of oral contraceptive pills (OCP) was a good risk factor for breast cancer. 78.18% of BC patients were using the OCP, while 21.82% did not used it. About the BBD patients the data showed that about 33.3% used OCP, and 66.7% neglected the OCP (fig. 6).

Patients and hormone replacement therapy

In fig: 7 (16.36%) breast cancer patients were taking HRT and 83.64% were not taking HRT, while for the BBD patients 8.3% were taking HRT and 91.7% were not taking HRT

Relation of BC to having family history

The higher number recorded for this risk factor in both BC and BBD patients were from patients who had no family history (55%, 75%) respectively. 29% and 8.33% of the patients had first degree family history relation for both BC and BBD patients respectively. In BC 9% had second degree, while 16.66% of BBD patients had second degree, regarding the third degree 7% of BC was found, while there was no record about third degree for BBD patients, (fig. 8).

Distribution of patients according to type and stage of disease

Breast cancer patients were 100% had invasive ductal carcinoma, first stage showed zero percentage, while in the second, third and fourth stages, the percentages were 25, 30, and 30% respectively, and 15% of them had unknown stage of BC. About the BBD patients 35% had unknown type of the disease, 30% had lipoma, 15% had fibroadenoma, 5% had fat necrosis, 10% had fibrosis, and 5% had duct ectasia shown in (fig. 9 and 10).

Body mass index and breast cancer

Body mass index is an important risk factor for BC. There were 40.45% of the BC patients had BMI≥ 30 which were obese females, while 40.63% of them had BMI (25- 29.9), 15.54% of the patients had BMI=18.5- 24.9, and only 3.63% of them had BMI< 18.5. In BBD patients 33.33% had obesity (BMI≥ 30), 45% of the examined patients had over weighted BMI (25-29.9), only 21.66% had BMI=18.5-24.9, and no patient has BMI< 18. While 80.78% of patients had BMI more than 25, which was considered as overweight and obese women as shown in (fig. 11).

Marital status of the patients

Fig: 12 showed 9% and 11.66% of breast cancer and BBD patients were single respectively, 78.18% and 76.66% of BC and BBD patients were married respectively, while 12.72% and 11.66% of BC and BBD patients were widowed respectively. Our results showed about 7.5% of patients were single, while all of the remaining was married.

Discussion

Age groups of the patients

Our study was in agreement with Montazeri *et al*. (2003) and Rennert (2009) which showed that the Iraqi BC patients had an age distribution that was nearly the same as that seen in Iran, Egypt and Jordan. In Sulaimanyiah- Iraq also agreed with our findings they found that currently diagnosed at advanced clinical stages with 60% of patients being under 50 years of age (Majid *et al*., 2010). In contrary with our study, in the United States, more than three- fourth of all breast cancers occurred in women aged 50 or older (Palmer *et al*., 2003). The greatest risk of BC occurs with increasing age, with incidence doubling every 10 years until menopause (McPherson *et al*., 2000). This increasing risk may be indicative of the lifetime accumulation of exposures to those risk factors, as well as genetic events throughout the lifetime (Alwan, 2010).

Patients and age at menarche

A woman who began menstruating before 12 years old has a 10- 20% increased risk of BC compared to one whose menstruation started when she was older than 14 years of age (Berkey *et al*., 1999). In addition, women who experience a delayed natural menopause (after the age of 55) are twice as likely to develop BC compared to women who experience menopause before the age of 45 (McPherson *et al*., 2000) which is in agreement with our study. Older age at menarche typically is reported to be associated with reduced BC risk, while older age at menopause is associated with increased risk. These relationships are believed to be mediated through estrogen production (Henderson *et al*., 1996).

Regularity of menstruation and patients

This study showed only one-fourth of the BC patients had irregular menstruation to the extent that irregular menstrual cycles reflect an ovulatory cycles, our findings support the hypothesis that the cumulative number of regular ovulatory cycles increases BC risk (den Tonkelaar and de Waard, 1996). Rockhill *et al.* (1996) found little support for the hypothesis that a longer time until onset of regular menstrual cycling was associated with reduced risk of BC, which is in contrast with our study. Parazzini *et al*. (1993) reported an increased risk among women who had menstrual cycles lasting 31 days or longer, but those women whose cycle was too irregular to estimate were at reduced risk.

Age at first pregnancy of the patients

Other reproductive events have also shown a linear association with risk for BC, specifically, women who gave birth for the first time before age 18 experience one- third the risk of women who have carried their first full- term pregnancy after age 20 (Yoo *et al*., 2002). Women who have their first full- term pregnancy at a relatively early age have a lower risk of BC than those who never have children or those who have their first child relatively late in life (Wohlfahrt and Melbye, 2001). There is also consistent evidence that first pregnancy completed before age 30- 35 lowers risk of BC and that first full- term pregnancy after age 30- 35 raises risk (Helmrich *et al*., 1983). It has been observed that five to seven years after pregnancy, women, especially older women are at increased risk of BC. This increase in risk is thought to be attributable to the increase in gestational hormone levels that occur during the first pregnancy (Bernstein, 2002 and Dumitrescu and Cotarla, 2005).

Patients and age at diagnosis

A 2- to 4- fold higher risk was found for women who experienced menopause after 50 years of age; meanwhile, the time from menarche to menopause is an interesting factor in considering its relation to the mechanism of breast carcinogenesis (Suh *et al*., 1996). Women who have menopause after the age of 55 years are twice as likely to develop BC as women who experience the menopause before the age of 45 years (Khalid *et al*., 2009). Furthermore, the increased cumulative exposure from the combined effect of early menarche and late menopause has been associated with elevated risk (Lipworth, 1995).

Relation between BC and oral contraceptive pills

Other study attempting to link oral contraceptives with increased BC have been inconclusive (Marchbanks *et al*., 2002). A case control study was done in Basrah in 2005 found that there was no association between the use of OCP and development of BC (Dahooz and Hawaz, 2005). Some studies suggest that past use of OCP may increase risk of BC in postmenopausal women, especially in those with a long history (more than 10 years) of OCP use (Van Hoften *et al*., 2000). The association of OCP use with BC is related to the duration,

dosage, pattern of usage, type of OCP and the age of first use (McPherson *et al*., 2000).

Patients and hormone replacement therapy

Use of estrogen replacement therapy is another factor associated with increased hormone levels and it has been found to confer a modest (less than two-fold), elevation in risk when used for 10-15 years or longer (Kelsey, 1993). The long- term (more than five years) use of postmenopausal estrogen therapy (ERT) or combined estrogen/ progestin hormone replacement therapy (HRT) may be associated with an increase in BC risk (Downing *et al*., 2007).

Relation of BC to having family history

Similar to our findings, others found most women who get BC (approximately 80 percent) have no such family history of the disease (Porch *et al*., 2002). There was an increased risk of BC among Kurdish patients who had a positive family history compared to case controls.

Distribution of patients according to type and stage of disease

According to the WHO classification (World health organization, 2008), the most common histological type determined microscopically was invasive ductal carcinoma. According to the AJCC system, the frequencies were 7.6%, 45.1%, 31.5% and 15.7% for stages I, II, III and IV respectively (Alwan, 2010). For patients with a designated stage, there was no significant relationship between tumor stage and age nor was the relationship significant when patients whose stage was unknown were included (Majid *et al*., 2010).

Body mass index and breast cancer

Some studies agreed with our results which they had shown that a high BMI was positively related with BC (Helmrich *et al*., 1983). A study done in Basrah in 2005 showed significant association between increase body mass index and risk of BC (Dahooz and Hawaz, 2005). Obesity is another known risk factor for BC Obese women have an increased risk for postmenopausal but not premenopausal BC. The association between obesity and BC risk has been proposed to be largely due to increased estrogenic activity in overweight women. Increased body weight results in elevated circulating estrogens from peripheral aromatization of androgensin adipose tissue. Breasts, which are estrogen- sensitive tissues, are therefore exposed to more estrogen stimulation in obese women, leading to an increased risk for BC (Kelsey, 1993 and McTiernan, 1997).

Marital status of the patients

Study by Ibarluzea *et al*. (2004) agreed with our results which documented that BC is common in married females. Other study also found higher ratio of BC among married females (Montazeri *et al*., 2003), while Pharoah *et al*. (1997) was disagree with our results which showed that never married women were at higher risk for BC which may be due to exposure of married women to many hormonal changes like parity, and this factor showed to be protective factors against breast cancer.

Conclusions:

- Age group (38- 47) was the most susceptible group for breast cancer.
- In breast cancer patients, some risk factors like (regularity of menstruation, age at diagnosis, BMI, age at first pregnancy, OCP and family history) were more prevalent than BBD.

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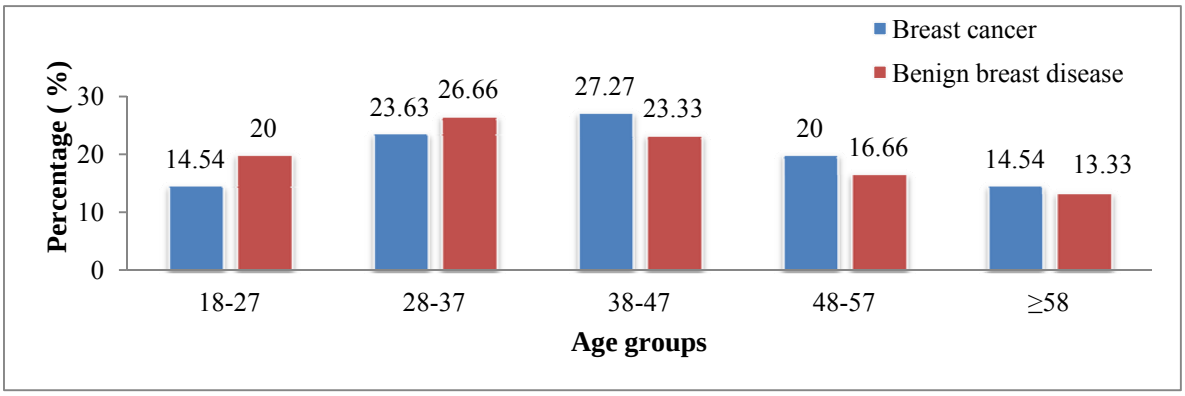

Figure (1): Age groups of the patients.

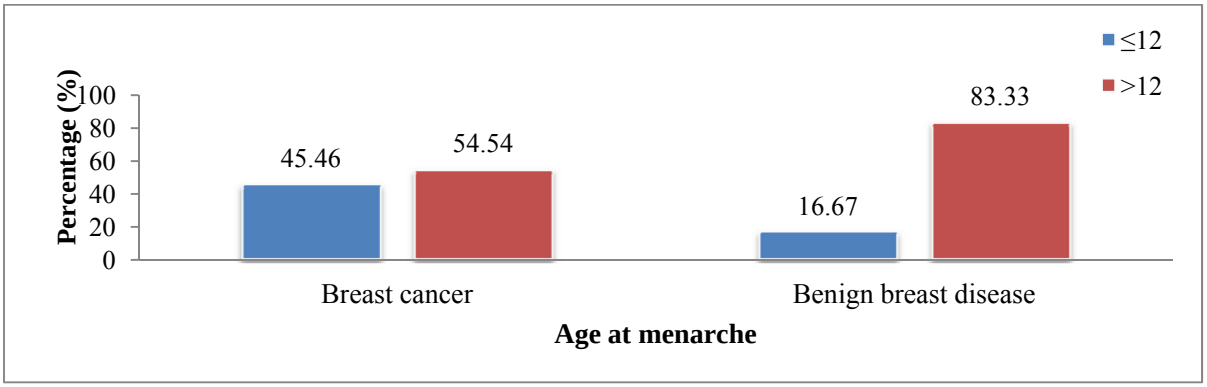

Figure (2): Patients and age at menarche.

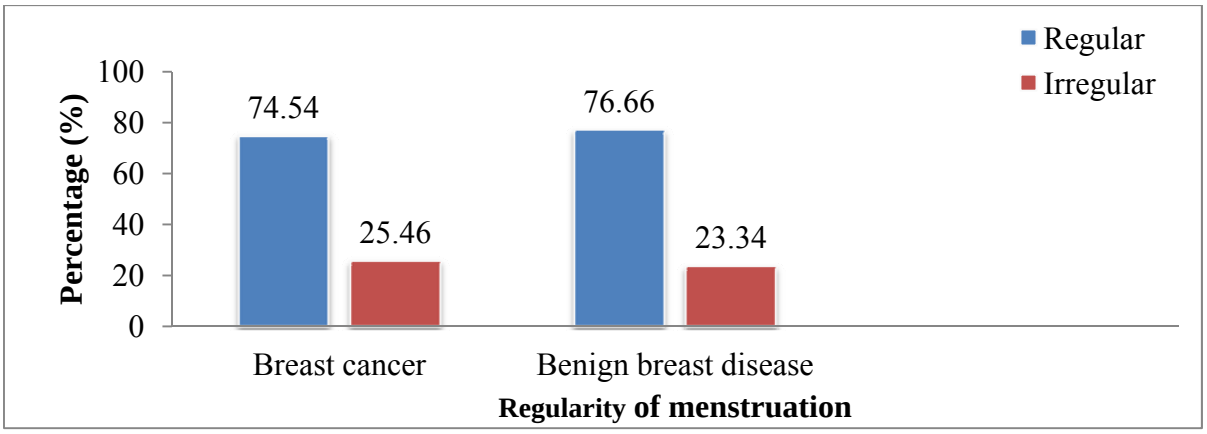

Figure (3): Regularity of menstruation and patients.

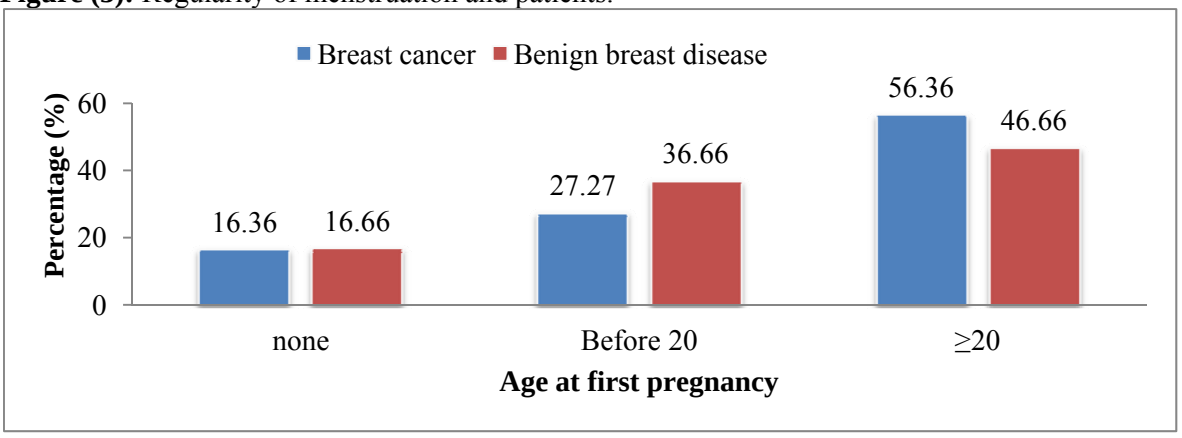

Figure (4): Age at first pregnancy of the patients.

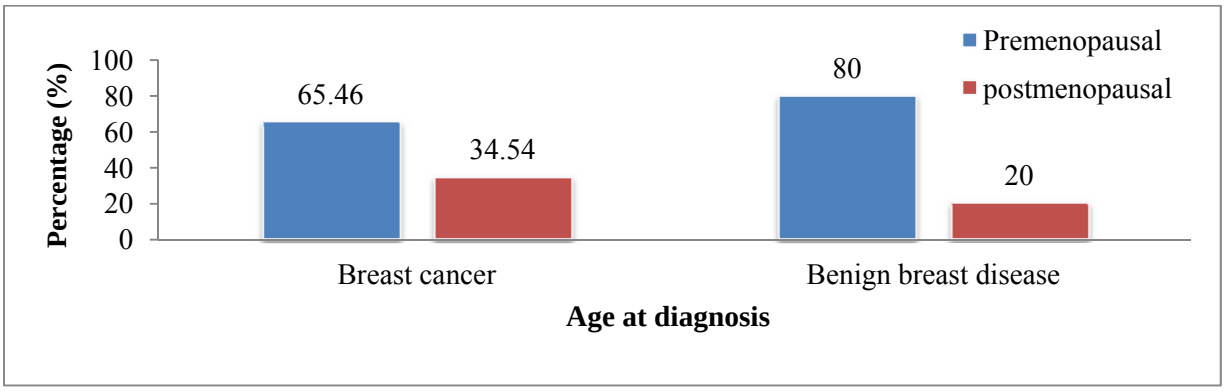

Figure (5): Patients and age at diagnosis.

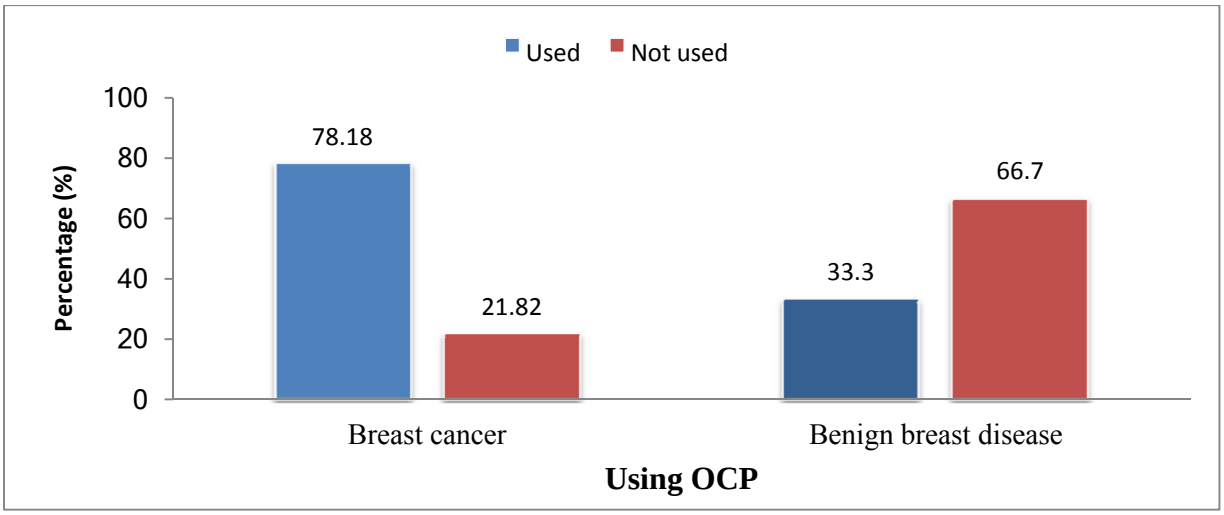

Figure (6): Distribution of patients according to using OCP.

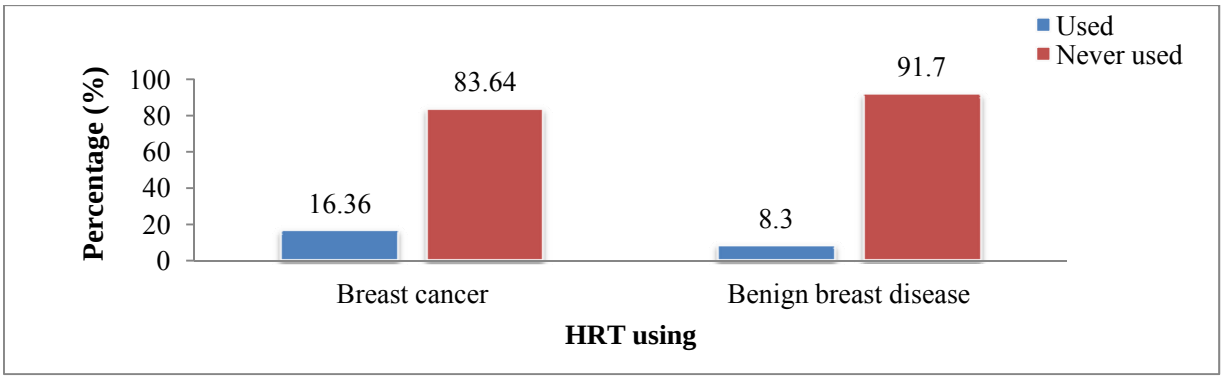

Figure (7): Patients and hormone replacement therapy.

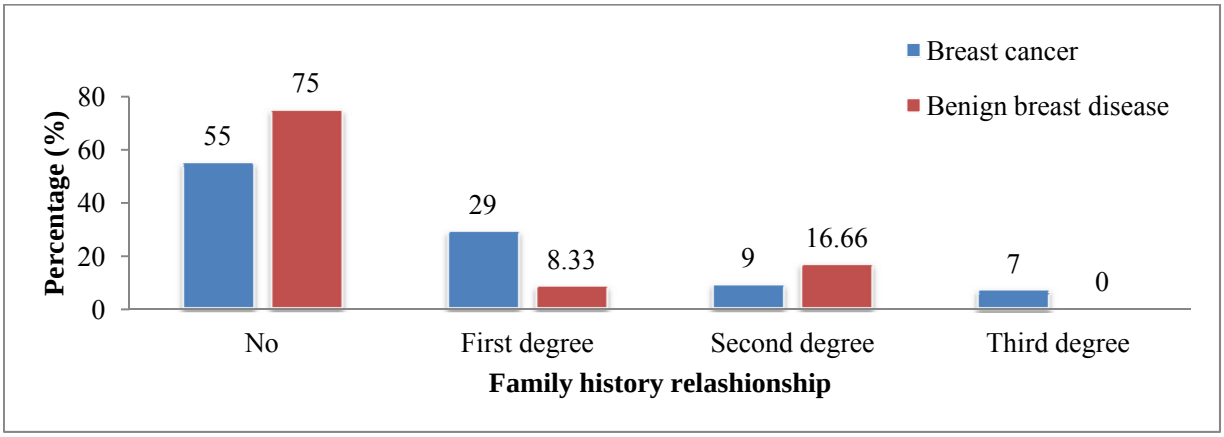

Figure (8): Percentage of patients according to family history relationship.

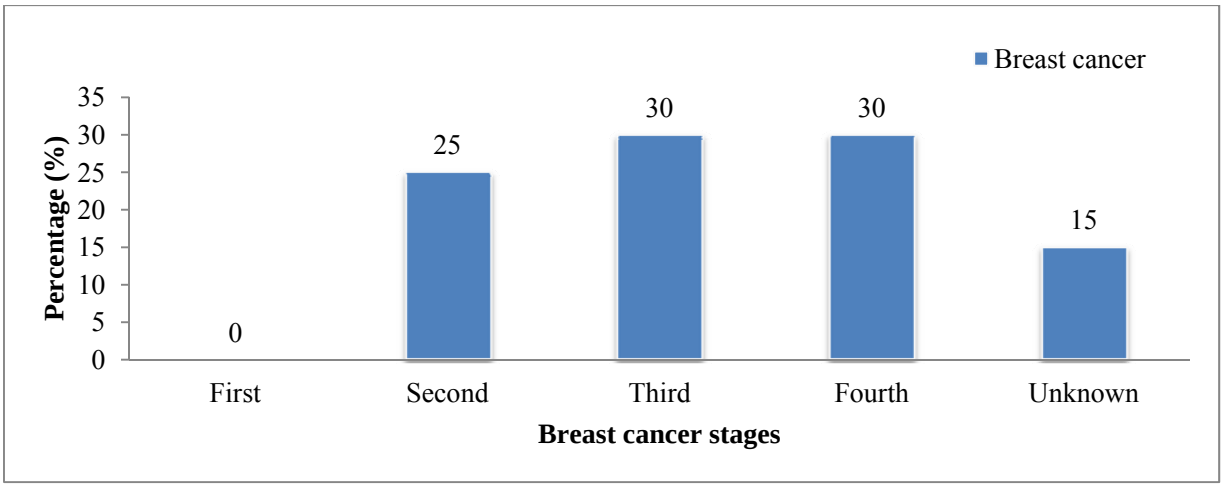

Figure (9): Percentage of stages of the infiltrative ductal carcinoma.

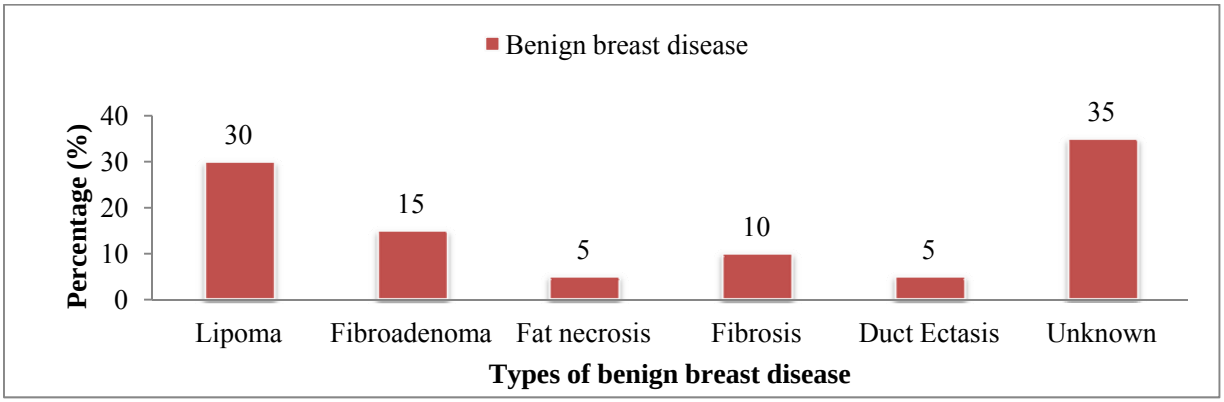

Figure (10): The percentage of types of BBDs.

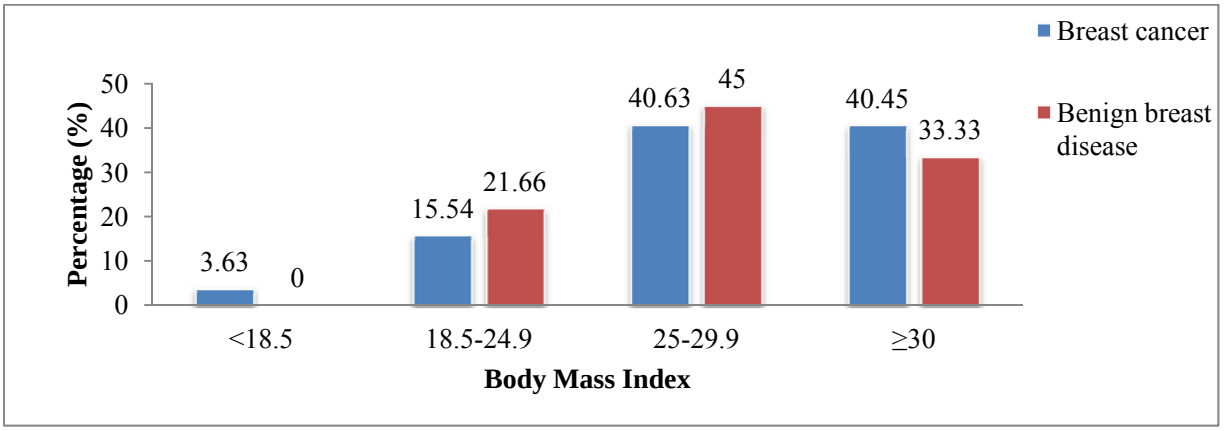

Figure (11): Patients and body mass index.

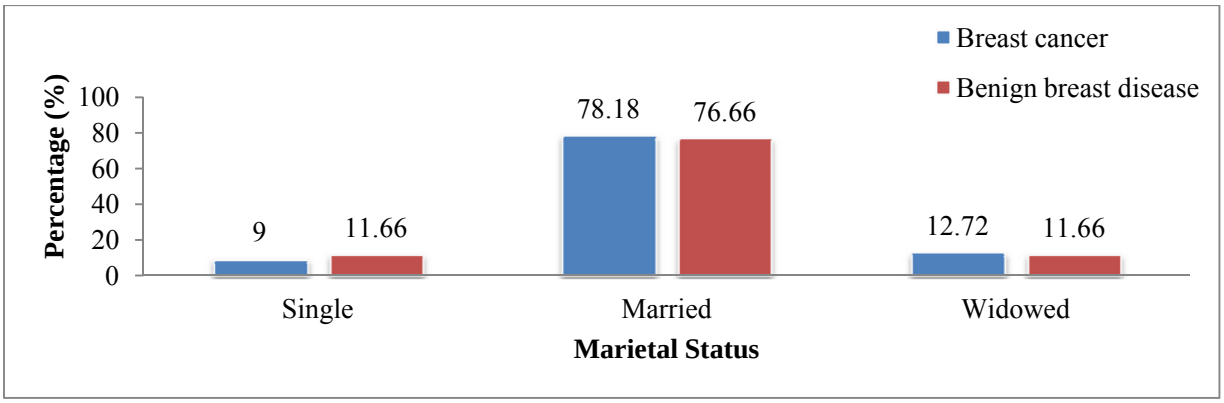

Figure (12): Percentage of the patients according to the marital status

دراسة بعض عوامل الخطورة فى السرطان الثدي قبل معالجة فى مدينة اربيل

الخلاصة

اجريت هذه الدراسة في مستشفى رزكاري و اربيل التعليمي في محافظة اربيل من الفترة آب ٢٠١٠ الى حزيران ٢٠١١ , شملت ١١٥ مريضة مصابة بورم الثدي (٥٥ ورم خبيث و٦٠ ورم حميد) مع ٤٠ امراة سليمة اعتبرن كمجموعة السيطرة.

أظهرت النتائج عدة علاقات بين عوامل الخطورة وسرطان الثدي ومن هذه العوامل العمر، حيث تشير الدراسة الى ان معظم الحالات كانت بين الفئة العمرية (٣٧–٤٨) سنة عند مقارنتها بالفئات العمرية الأخرى. عدد المريضات اللاتي بدأن الدورة الشهرية بعد ١٢ سنة كان أعلى مقارنة بالنساء اللاتي بدأن عند أو قبل ١٢ سنة. اظهرت النتائج ان النساء اللاتي لديهن الدورة الشهرية بشكل منتظم اظهرت أعلى ارتفاع عند مقارنتهن بالنساء اللاتي لديهن الدورة الشهرية غير منتظمة. كذلك النساء اللاتي ولدن اول طفل عند سنة ٢٠ او اكثر شكلن اعلى نسبة اصابة بالسرطان مقارنة ببقية النساء. النساء اللاتي يستخدمن حبوب منع الحمل كان لهن اعلى نسبة اصابة بسرطان الثدي مقارنةبالاصابة بالاورام الحميدة بينما النساء اللاتي لم يستخدمن معالجة بديلة بالهرمون سجلن أعلى نسبة مقارنة بالنساء اللاتي يستخدمن هذه المعالجة. واظهرت نصف المريضات لهن تاريخ عائلي للمرض.

لێکۆڵینەوەی هەندێك لە هۆکارە مەترسیەکانی شێرپەنجەی مەمك پێش چارەسەر کردن لە شاری هەولێر

پوختە

ئەم توێژینەوەیە لە نەخۆشخانەی ڕزگاری و نەخۆشخانەی هەولێری فێرکاری لە شاری هەولێر ئەنجامدراوە لە نێوان (ئاب ٢٠١٠- حوزەیران٢٠١١). کۆی ١١٥ ئافرەت وەرگیراوەبە شێوەیەك (٥٥ نەخۆشی شێرپەنجەی مەمك (زیان بەخش) , ٦٠ نەخۆشی گرێی مەمکی خاوێن(بێ زیان), وە ٤٠ ئافرەت وەك کۆنترۆل وەرگیراوە.

ئەنجامی ئەم توێژینەوەیە دەریخست کەوا پەیوەندیەکی بەهێز هەیە لەنێوان هۆکارەکانی مەترسی توشبوونی شێرپەنجەی مەمك, یەکێك لەم هۆکارانە تەمەنە, دەرکەوت کەوا زۆربەی توشبوانی شێرپەنجەی مەمك لە نێوان تەمەنی(٣٨-٤٧) سالّی بوون بە بەراورد لەگەل تەمەنەکانی تر. ڕێژەی توشبوان بە نەخۆشی شێرپەنجەی مەمك لەو ئافرەتانەی کەدەگەنە هەرزەکاری (دەستپێکی سووڕی مانگانە) ڕێژەی توشبوون دوای ١٢ سالّی زیاترە لەو ئافرەتانەی کە هەرزەکاریان لە ١٢ سالّی یان زووتر. دەست پێدەکات. نەخۆشی شێرپەنجەی مەمك لەو ئافرەتانەی کە سووڕی مانگانەیان ڕێك و پێکە زیاترە لەوانەی سووڕی مانگانەیان ناڕێکە. ڕێژەی توشبوون لەو نەخۆشانەی کەوا یەکەم منداڵ یان سکپڕی تەواویان تەمەنی ٢٠ سالّی یان گەورەترەوە بووە زیاترە لەو نەخۆشەکانی تر. ئەو ئافرەتانەی کەوا حەبی دژی سکپڕیان بەکارهێناوە زۆرترین ڕێژەیان هەبووە بۆ توشبوون بە شێرپەنجەی مەمك, بە بەراورد لەگەل گرێ ی مەمکی خاوێن. ئەو ئافرەتانەی کەوا چارەسەری هۆرمۆنیان وەرنەگرتووە زۆرترین ڕێژەیان تۆمار کردووە بە بەراورد لەگەل ئەو ئافرەتانەی کەوا ئەم چارەسەریانەیان وەرگرتووە. نیوەی توشبووان مێژووی شێرپەنجەی مەمکیان هەبووە لە خێزاندا.

ژمارەی نەخۆشەکانی توشبوو بە شێرپەنجە لە قۆناغی سێ یەم و چوارەم زیاتر بوو وەك لە قۆناغی یەکەم و دووەم وقۆناغ نەزانراوەکان. دەربارەی توشبوانی گرێ ی مەمکی خاوێن, هۆکاری توشبوونی نەزانراو زۆرترین ڕێژەی تۆمارکردووە.

DIVERSITY OF SOIL MICROFUNGI IN PINE FOREST AT DUHOK GOVERNORATE, KURDISTAN REGION, IRAQ

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Abstract:

The soil microfungi inhabiting pine (*Pinus brutia* Tern.) forests in Atrush and Zawita, Duhok governorate(Kurdistan region),Iraq were examined by using five isolation methods with the objective to compare the diversity of soil microfungi at O1 and O2 soil horizon layers from both sites. A total of 26 genera and 51 species of soil fungi as well as yeasts and non- sporulating mycelia were isolated from the two sites. *Aspergillus* was represented by 17 species and thus showed the widest diversity among all recovered genera. *A.niger* and *A.fumigatus* were the most frequent species among the genus. Black aspergilli were the most common species among the genus. They were represented by five species *viz A.awamori, A.foetidus, A niger, A. sclerotioniger* and *A.vadianes. Penicillium* was the second in the number of species isolated and was represented by 6 species. These include *P. cammeberti, P. citrinum, P. coryophilum, P. digitatum , p. glabrum* and *P. roquefotri.*. Two species were identified in each of the genera *Absidia, Cladosporium, Emericella, Mucor and Paecilomyces*. The rest genera that included *Alternaria, Cunnighamella, Chaetomium, Corynascus, Eupenicillium, Fusarium, Gymnoascus, Melanocarpus, Monilia, Pestatiolopsis, Phaeoacremonium , Phialophora, Rhizopus, Sordaria, Stachybotrys, Scytalidium, Thermomyces, Trichoderma* and *Ulocladium* were each represented by one species. The highest similarity index for fungal community inhabiting soil at both sites was found in soil at O1 layer (Atrush) and O2 layer (zawita).The least (SI) was recorded between O1 layer (Zawita) and O2 layer (Atrush). *Absidia spinosa*, and *Mucor plumbeus* are newly recorded from Iraq; hence they represent new additions to the Iraqi mycobiota.

Keywords: microfungi, diversity, forest soil, Iraq.

Introduction

Soil is the habitat for living many organisms including, bacteria, fungi, algae, viruses and protozoa. It supports the growth of a variety of plants, animals and soil microorganisms (Chiacek *et al*., 1996).

Soil fungi play an important role as a major decomposers in the soil ecosystem. They also provide mankind with very useful pharmaceutical products, such as antibiotics and other valuable substances, including organic acids, enzymes, pigments and secondary metabolites used in the food industry and fermentation. In addition many soil fungi are biological control agents for plant pathogens and insect pests. On other hand , some of them are very harmful causing food spoilage and diseases to plants, animals, and humans (Manoch, 2004).

Despite the fact that several studies on Iraqi soil there were carried in different ecosystems, very little is known regarding their diversity in forest soil. Previous studies included desert soil (Abdullah *et al*., 1986), soil at date palm plantations (Abdullah and Zora, 1993), cultivated soil in middle and Southern parts of Iraq (Al-Doory *et al*., 1959; Ismail and Abdullah, 1977; Abdullah and Al-Bader(1989, 1990), forest soils (Al-Bader *et al*., 2000; Abdullah and Abdullah, (2008, 2009, 2010) and from soil at vineyards (Abdullah and Mohammed, 2011).

The present study was carried out on soil fungi present in Atrush-Zawita natural pine forest in Duhok governorate to study species abundance, diversity and taxonomy.

Materials and Methods

Site Description

The study was conducted in the unique natural pine forest restricted to Zawita-Atrush locality (36°52¯ - 36°90¯ N latitude and 43° 17¯ -43° 58¯ E longitude). The forest supports pure stand of *Pinus bruitia* Tern trees covering a mountainous area of 100 Km square, 10 Km N.W of Duhok city (Townsend and Guest, 1966; Shahbaz, 2007).

Both sites were about 8 Km apart from each other and have similar climatic conditions, the average minimum temperature is 8.97c° and average maximum temperature is 22.34 c°. The total rainfall average is 578.5mm. The monthly average relative humidity (RH%) is ranging between 18.5% to 68% with a total average 44.18% (Duhok metrological station, 2005).

The soil type is belonging to the mountain soil according to the classification of Buringh (1960) that constitute the bulk of soil in Atrush-Zawita forest. Top soil is alkaline and mostly covered by individual tress of *Pinus bruitia*. The trees found to live up to 1600m in Zawita region (Shahbaz *et al*., 2002).

Collection of Soil Samples

A total of 20 samples were collected during November, 2011 from the two sites, 10 samples points were selected in each site (Atrush-Zawita). Soils were taken from the O1 horizon layer with sterile trowel for each location in each site (Atrush and Zawita) after first removing the litter layer (L1-L2). Similarly soil samples were collected from the O2 horizon layer after removing the O1 horizon layer. Afterwards, soil samples were stored in polythene bags at 5C° and were processed within 1-2 weeks after collection.

Isolation of Fungi from Soil

Five isolation methods were used: dilution plating method (Parkinson et *al*. 1971), direct plating method (Warcup, 1960), soil treatment with 5% Acetic acid (Furuya and Naito, 1979), treatment with 70% ethanol (Warcup and Baker, 1960), and treatment with 2% phenol (Furuya and Naito, 1980). Two types of media were used to isolate fungi: potato carrot agar (PCA) (20g peeled potato, 20g carrot, 20g agar, 1L distilled water) and malt extract agar (MEA). Media were supplemented with 50mg/L chloramphenicol.

Identification of Fungi

Identification of fungal isolates was based on morphological and cultural characteristics. General and specific taxonomic literature was used for identification of fungal species. Domsch *et al*., (1980); Klich (2002), Ellis (1971, 1976), Pitt and Hocking (1997), Watanabe (2002), Samson *et al.*, (2007), Frisvad and Samson (2004).

Data Analysis

Frequency of occurrence for soil samples was calculated based on the following formula:

$$\%FO = \frac{number\ of\ soil\ samples\ that\ a\ particular\ fungal\ species\ observed}{total\ number\ of\ soil\ samples} \times 100$$

Comparing the similarity of fungal species composition between different habitats, Sorensen´s index (SI) was applied (Sorenson, 1948). The index was calculated as the following formula

$$\mathbf{SI} = \frac{2c}{a+b}$$

Where

a = total number of species at site 1

b = total number of species at site 2

c = number of species common to both sites.

Similarity is expressed with values between 0 (no similarity) and I (absolute similarity).

Results

A total of 32 species belonging to 14 genera including yeasts and non sporulating mycelia were isolated from soil of O1 layer obtained from the two sites (Zawita and Atrush). Their frequency of occurrence is presented in table 1.

Twenty six species were detected on PCA medium and 32 species developed on MEA medium after incubation at 25ºC.

Aspergillus was represented by 12 species and thus showed the widest diversity among all recovered genera. *A.niger* and *A.fumigatus* were the most frequent species within the genus.

Black Aspergilli were the most common species among the genus. They were represented by five species *A. awamori, A.foetidus, A.niger, A.vadiances* and *A. sclerotioniger*.

Penicillium was second in the number of species isolated and was represented by 3 species. These include *P. camemberti, P. citrinum* and *P. glabrum*. The later species was the most frequent among the genus.

Two species were indicated for each of the genera *Mucor* and *Paecilomyces*. The rest genera that included *Alternaria, Cheatomium, Cladosporium, Fusarium, Gymnoascus, Monilia, Rhizopus, Sordaria, Stachybotrys, Ulocladium* were represented by one species each.

Twenty two species were found common to both sites (Zawita and Atrush). *Aspergillus alliaceus, A.ornatulus* and *Gymnoasus ressii*were detected only from Zawita sites whereas, *Mucor* sp., *Stachybotrys atra* and *Cladosporium gallicola* were detected from soil at Atrush site.

Table (1): % Occurrence of Fungi in Soil from O horizon (O1) Layer Incubated at 25ºC

	ZAWITA		***ATRUSH***	
FUNGAL SPECIES	**% Occurrence / on PCA 25℃**	**% Occurrence / on MEA 25℃**	**% Occurrence / on PCA 25℃**	**% Occurrence / on MEA 25℃**
A.awamori Nakaz	10%	20%	40%	30%
A. aliaceus Thom	-	10%	-	-
A.flavus Link	30%	20%	-	10%
A.foetidus Thom&Raper	10%	10%	20%	20%
A.fumigatus Fresen	70%	80%	60%	90%
A.niger Fresen	80%	90%	90%	100%
A.ochraceus K.Wilh	20%	20%	-	20%
A.ornatuluns Samson&W.Gams	-	10%	-	-
A.tamarii Kita	10%	10%	20%	-
A.terrus Thom	10%	30%		10%
A.vadiances Samson, de Vries, Frisvad & Visser	20%	40%	10%	-
A.sclerotioniger Samson & Frisvad	-	30%	-	30%
Alternaria alternata (Fr.) Keissler	10%	-	30%	10%
Cheatonium sp.	-	10%	20%	
Cladosporium gallicola Sutton	-	-	10%	20%
Fusarium sp.	10%		30%	40%
Gymnoascus ressii Baranetzky	-	10%	-	-
Monillia sp.	30%	40%	30%	10%
Mucor sp.	-	-	10%	10%
Mucor plumbeus Bonordon	-	30%	-	20%
P.camemberti Thom	10%	40%	10%	10%
P.citrinium Thom	-	30%	20%	20%
P.glabrum (Wehmer)Westling	40%	70%	20%	50%
Paecilomyces variotii Bainier	-	50%	40%	-
Paecilomyces sp.	40%	30%	10%	-
Rhizopus sp.	20%	60%	40%	50%
Sordaria fimicola(Roberg ex Desm.)Ces De Not.	-	10%	20%	40%
Stachybotrys atra Corda	-	-	20%	30%
Sterile mycelium(white)	40%	60%	30%	70%
Sterile mycelium(brown)	20%	30%	20%	30%
Ulocladium atrum preuss.	10%	-	10%	30%
Yeast	40%	60%	50%	40%

Table (2): % Occurrence of Fungi in Soil from O horizon Layer (O2) Incubated at 25ºC

FUNGAL SPECIES	ZAWITA		ATRUSH	
	% Occurrence / on PCA 25℃	% Occurrence / on MEA 25℃	% Occurrence / on PCA 25℃	% Occurrence / on MEA 25℃
A.awamori Nakaz	30%	50%	50%	40%
A.alliaceus Thom	-	30%	-	20%
A.carbonarius (Bainier)Thom	-	50%	-	30%
A.flavus Link	30%	30%	10%	20%
A.foetidus Thom&Raper	40%	50%	40%	30%
A.fumigatus Fresen	70%	90%	80%	100%
A.niger Tiegh.	80%	100%	50%	60%
A.ochraceus K.Wilh.	30%	50%	30%	30%
A.ornatulas Samson&W.Gams	-	20%	-	-
A.oryzae Ahlburg)Cohn.	-	-	-	20%
A.tamarii Kita	10%	20%	-	10%
A.terrus Thom	-	40%	30%	-
A.vadiances Samson,de Vries,Frisvad&Visser	-	50%	-	30%
A.sclerotioniger Samson&Frisvad	20%	30%	-	20%
Absidia spinosa Lender	20%	-	-	10%
Alternaria alternate (Fr.)Keissler	-	10%	-	-
Cheatonium sp.	40%	30%	20%	20%
Canninghamella echinulata (Thaxt.)Thaxt.	20%	-	-	-
Emericella nidulans (Eidam)Vuill.	10%	10%	-	-
Eupenicilium sp.	30%	90%	50%	100%
Fusarium sp.	40%	-	50%	20%
Gymnoascus ressii Baranetzky	10%	10%	-	-
Monillia sp.	40%	40%	30%	40%
Mucor sp.	20%	10%	20%	10%
Mucor plumbeus Bonordon	10%	10%	-	
P.digitatum (Pers.)Sacc.	10%	20%	-	10%
P.camemberti Thom			30%	20%
P.citrinium Thom	30%	20%	-	20%
P.glabrum (Wehmer)Westling	40%	40%	30%	40%
P.roquiforti Thom	10%	-	-	10%
Paecilomyces variotii Bainier	-	30%	-	10%
Paecilomyces sp.	40%	10%	-	-
Rhizopus sp.	40%	30%	50%	40%
Sordaria fimicola (Roberg ex Desm)Ces De Not.	40%	20%	-	30%
Stachybotrys atra Corda	-	30%	10%	30%
Sterile mycelium(white)	40%	50%	40%	100%
Sterile mycelium(brown)	-	-	-	20%
Ulocladium atrum Preuss.	10%	-	-	20%
Yeast	-	30%	50%	60%

Frequency of occurrence of fungi detected from the O2 layer at both sites is showed on Table 2. A total of 39 species including yeasts and non-sporulating mycelia assigned to 17 genera were detected after incubation at 25C°. *Aspergillus* showed the widest diversity and was represented by 14 species. *A.niger, A.fumigatus, A,awamori, A.foetidusand A.ochraceuss* were the most frequent species detected on both media. *Penicillium* was second and represented by five species among them. *P. glabrum* was the most frequent.

A.ornatulus, Alternaria alternate, Cunnighamella echinulata, Emericella nidulaus, Gymnousus ressii, Mucor plumbeus and *paecilomyces* sp., were isolated from Zawita soil, whereas, *A.oryzae* and *P. cammeberti* were detected in the soil at Atrush site.

Table 3 shows the data for % frequency of occurrence of fungi isolated from O1 layer on both PCA and MEA media after incubation at 40 C°.

A total of 31 species assigned to 16 genera including yeasts and non-sporulating mycelia were detected from both sites.

Aspergillus was represented by 12 species among them, *A.niger, A fumigatus* were the most frequent and showed high percentage of occurrence. *Pinicillium citrinum* was the only species isolated at 40ºC.

A. candidus, A. tamari, Cheatonium sp, Corynoascus spedonium, Melanocarpus sp, P. citrinium, Paecilomyces sp., Sordaria fimicola, and *Stachybotrys atra*, were isolated from Atrush site whereas *A. flavus, A. ochraceus, A. ornatulans, C. gallicola* and *G ymnoascus ressii* were detected in Zawita sites.

Table (3): % Occurrence of Fungi in Soil from O horizon Layer (O1) Incubated at 40c°

FUNGAL SPECIES	ZAWITA		ATRUSH	
	% Occurrence / on PCA 40℃	% Occurrence / on MEA 40℃	% Occurrence / on PCA 40℃	% Occurrence / on MEA 40℃
A.awamori Nakaz	70%	20%	-	10%
A.candidus Link	-	-	40%	-
A.carbonarius(Bainier)Thom	-	40%	-	10%
A.flavus Link	20%	10%	-	-
A.foetidus Thom&Raper	10%	50%	-	10%
A.fumigatus Fresen	30%	80%	50%	50%
A.niger Tiegh	100%	100%	90%	80%
A.ochraceus K.Wilh	30%	30%	-	-
A.ornatulus Samson&W.Gams	-	20%	-	-
A.tamarii Kita	-	-	-	40%
A.terrus Thom	-	10%	50%	40%
A.vadences Samson, de Vries,Frisvad&Visser	20%	-	10%	10%
Cheatomium sp.	-	-	-	40%
Cladosporium gallicola Sutton	20%	30%	-	-
Corynascus sepdonium(C.W.Emmons)Arx	-	-	10%	10%
Emericellanidulans(Eidam)Vuill	-	10%	60%	30%
Fusarium sp.	20%	-	-	10%
Gymnoascus ressii Baranetzky	-	10%	-	-
Melanocarpus sp.	-	-	20%	-
Monillia sp.	30%	10%	-	80%
Mucor sp.	-	10%	20%	70%
P.citrinium Thom	-	-	-	30%
Paecilomyce svariotii Bainier	20%	30%	-	20%
Paecilomyces sp.	-	-	-	10%
Rhizopus sp.	-	70%	60%	70%
Sordaria fimicola(Roberg ex Desm.)Ces De Not	-	-	-	60%
Stachybotrys atra Corda	-	-	30%	30%
Sterile mycelium(white)	10%	30%	40%	40%
Sterile mycelium(brown)	-	10%	-	-
Trichoderma sp.	-	-	10%	10%
Yeast	30%	-	-	40%

Table 4 shows the data for the % occurrence of fungi isolated from O2 layer on both media after incubation at 40ºC from both sites.

A total of 25 species distributed in 14 genera in addition to yeasts and non-sporulating mycelia were isolated. *Aspergillus* was the most frequent genus and showed the highest diversity. The genus was represented by 10 species among them A. *niger, A. fumigatus, A. awamori,* were the most frequent species. *Paecilomyces* was represented by two species the rest genera were represented by one species each.

A.candidus, A. ochraceus, A. terrus, C. sepedonium, E. nidulans, and *Trichoderma* sp, were detected in Atrush site, whereas, *A .flavus, Mucor plumbues, Paecilomyces* sp., and *Pestalotiopsis* sp., were isolated from Zawita site.

The majority of species were found common to both O1 and O2 soil layers except for *C. gallicola, E. rugulosa, Melanocarpus* sp, and *Pestalotiopsis* sp. were detected in O2 soil layer.

Corynascus sepedonium, Melanocarpus sp, and *Pestatiolopsis* sp, were not detected at 25ºc on both media.

Similarity index (SI) for soil fungal community in O1 and O2 horizon layers at Atrush site was higher (0.914) than that calculated for the two layers of O horizon (0.845) at zawita site.

The highest similarity index (0.929) was found between soil fungal communities from O1 layer at Atrush site with O2 layer at Zawita site. The least (SI) was found between O1 layer at Zawita site and O2 layer at Atrush site (Table 5).

Table (4): % Occurrence of Fungi in Soil from O horizon Layer (O2) Incubated at 40ºC

	ZAWITA		***ATRUSH***	
FUNGAL SPECIES	**% Occurrence / on PCA 40℃**	**% Occurrence / on MEA 40℃**	**% Occurrence / on PCA 40℃**	**% Occurrence / on MEA 40℃**
A.awamori Nakaz	70%	60%	-	40%
A.candidus Link	-	-	20%	-
A.carbonerius (Bainier)Thom		30%	-	20%
A.flavus Link	-	40%	-	-
A.foetidus Thom&raper	30%	80%	-	20%
A.fumigatus Fresen	80%	70%	60%	100%
A.niger Fresen	100%	100%	90%	100%
A.ochraceus K.Wilh.	-	-	-	20%
A.terrus Thom	-	-	20%	30%
A.vadiances Samson,de vries,frisvad&Visser	30%	30%	10%	10%
Absidia spinosa Lender	40%	40%	10%	-
Corynascus sepdonum(C.W.Emmons)Arx	-	-	10%	-
Emericella nidulans (Eidam)Vuill	-	-	20%	-
Monillia sp.	90%	80%	-	30%
Mucor sp.	-	30%	20%	20%
Mucor plumbeus Bonordon	30	30%	-	-
P.camemberti Thom	-	-	-	10%
P.citrinium Thom	30%	40%	-	20%
Paecilomyces variotii Bainier	20%	50%	-	60%
Paecilomyces sp.	40%	-	-	-
Pestatiolopsis sp.	30%	30%	-	-
Rhizopus sp.	30%	30%	40%	50%
Sordaria fimicola (Roberg exDesm)Ces De Not.	-	70%	-	40%

Stachybotrys atra Corda	70%	-	30%	30%
Sterile mycelium(white)	-	70%	40%	-
Sterile mycelium(brown)	-	30%	-	50%
Trichoderma sp.	-	-	10%	-
Yeast	40%	100%	-	30%

Table (5): Similarity index (SI) between Soil Fungal Communities at Zawita and Atrush Sites

Sites	Zawita Soil O1	Zawita Soil O2	Atrush Soil O1	Atrush Soil O2
Zawita **Soil** O1	----------			
Zawita **Soil** O2	0.864	----------		
Atrush **Soil** O1	0.845	0.929	----------	
Atrush **Soil** O2	0.739	0.849	0.914	----------

Discussion

Among the *Aspergillus, A. fumigatus, A. niger, A. awamorri, A. foetidus* were the major species according to their frequency of occurrence in both sites. *A .fumigatus* and *A. niger* have been reported as the most frequent isolates from soil of date palm plantation in Iraq and among the most frequent isolates known from the other soils in arid region (Moubasher and Mustafa, 1970; Halwagy *et al*. 1982: Abdullah *et al*., 1986; Abdullah and Zora, 1993 Abdullah *et al*., 2007). This is primarily related to their high tolerance to both relatively high temperature and drought conditions (Durell and shields, 1960; Christensen, 1969). *A. awamori* and *A. foetidus* have been recently reported among the most common species inhabiting soil (particularly vineyard soil) in Kurdistan region (Abdullah and Abdullah, 2009; Abdullah and Mohammed, 2011).

Five species of *Penicillium* found in our study, *P.citrinium* was the most common one. The fungus is cosmopolitan species which has been frequently reported from a variety of habitats (Domsch *et al.*, 1980).

Only twenty eight species isolated from plates incubated at 40ºC . *A. fumigatus, A .niger, A. terreus, A. candidus, Emericella* spp., *Paecilomyces variotii* and *Thermomyces* sp., were previously reported from Iraq as a thermotolerant species (Abdullah and Al-Bader, 1990). According to Cooney and Emerson (1964), definition of thermophilic and thermotolerant fungi, the above reported species are considered thermotolerant species, since they grow at temperature with optimum between 30–40 ºC.

Emericella nidulans and *E. rugulosa*, two soil borne ascomycetes were isolated in our study at 40 ºC. The former species was previously reported in several occasions from soil in Southern Iraq and from sediments of Shatt–Al Arab river and southern Marshes (Abdullah and Zora, 1993; Abdullah and Abbas, 2008; Abdullah *et al*., 2010). Moreover, the two species have been recently reported from soil in Kurdistan region of Iraq (Abdullah and Abdullah, 2009).

The Ascomycete isolate which identified as *Melanocarpus* detected at 40 ºC is very close to *M. thermophilus* which was reported by Guarro *et al*., (1996) from soil at Nineva forest, North Iraq.

Sordaria fimicola is a common coprophilous fungus. However, the fungus has been repeatedly isolated from soil (Domsch *et al*., 1980, Furuya and Naito, 1979; Abdullah *et al*., 2010) and perhaps originated from disintegrated dung pellets incorporated into soil.

Our results for fungal isolation from soil in pine forest of Zawita and Atrush were compared with those of studies carried out on date palm plantation and forest soil in Iraq. There were

obvious differences among species composition for genera with darkly pigmented mycelium as well as for ascomycetes. Dematiaceous genera reported previously from soil in Iraq (Al-Doory *et al*., 1959; El-Dohlob and Al-Helfi, 1982; Abdullah and Zora, 1993; Al – Bader *et a*l., 2000) showing a high species diversity were *Alternaria* (5 species), *Curvularia* (3 species), *Drechslera* (5 species), *Ulocladium* (4 species), *Stemphylum* (3 species), whereas, *Alternaria* and *Ulocladium* were each represented by one species in the soil of pine forest. Other dematiaceous were not detected during our study. It has been suggested that fungi with darkly pigmented mycelia are well adapted to withstand intense higher radiation (Durell & Shield, 1960; Ranzoni, 1968), hence, low diversity level among dematiaceous taxa was due to the fact that forest soil at Zawita and Atrush is receiving low solar radiation because of the density of pine trees.

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جۆرێن (کەڕوێێن هویر) د ئاخا دارستانێن کاژان دا ل پارێزگەها دهوکێ – هەرێما کوردستانا عێراقێ

پوختە:

ڤەکۆلین ل سەر (کەڕوێێن هویر) د ئاخا دارستانێن کاژان ل ئەتروش و زاویتە ل پارێزگەها دهوکێ باکۆرێ عێراقێ هاتە کرن ئەوژی ب کارئینانا پێنج ڕێێن دابڕینا (کەڕو) ئەوژی ژبو بەراوردکرنا جۆرێن (کەڕوێێن هویر) د قاتێن ئاخێ دا ل هەر دو جهێن ناڤبری. (٥١) جۆرێن فگریاتان هاتنە دابڕین و دەست نیشان کرن ئەوژی دزڤڕنە بۆ (٢٦) ڕەگەزان زێدەباری (کەڕو و داڤێن کەڕوێی) ئەوێن نە پیس. ڕەگەزێ (اسبر جلس) دیاربو کو بلندترین جۆرە کو دبنە (١٧) جۆر . ئەو جۆرێن ڕەنگ ڕەش ژ ڕەگەزێ (اسبر جلس) ژ هەمویان بەربەلاڤ تڕە. ئەوژی (٥) جۆرن (*A.awamori, A.foetidus, A niger, A. sclerotionigerandA.vadianes.*

بەلێ ڕەگەزێ (*Penicillium*) ل ریزا دووێ هات پشتی (الاسبر جلس) کو ئەڤەژی دبیتە (٦) جۆر (و *P. cammeberti, P. citrinum, P. coryophilum, P. digitatum , p. glabrum P. roquefotri*

لێ ڕەگەزێن (*Absidia, Cladosporium, Emericella, Mucor and Paecilomyces*) هەرئێك ژ وان دبنە (٢) جۆر . بەلێ ڕەگەزێن دی هەمو کو ئەڤەنە (*Alternaria, Cunnighamella, Chaetomium, Corynascus, Eupenicillium, Fusarium, Gymnoascus, Melanocarpus, Monilia,Pestatiolopsis, Phaeoacremonium , Phialophora, Rhizopus, Sordaria, Stachybotrys, Scytalidium, Thermomyces, Trichoderma,Ulocladiu*

ب تنێ دبنە ئێك جۆر. بلندترین کۆمەلا فگریاتێن کو پتری یا وان وەکهەڤ ئەوبون یێن دناڤبەرا قاتێ ((zawita) O2 دگەل O2 (Atrush)هاتینە تومارکرن . بەلێ ئەوێن دی یێن د کێمترین ئالی دا وەکهەڤ ئەو بون یێن دناڤبەرا ((zawita) O2 دگەل O2 (Atrush)) هاتینە توامرکرن . هەردو جورێن *Absidiaspinosa,Mucorpleumbeus* ژبو جارا ئێکێ نە ا عیراقێ دهێنە تومارکرن .

تنوع الفطريات الدقيقة في تربة غابات الصنوبر في محافظة دهوك/ اقليم كوردستان العراق

الملخص:

تم دراسة الفطريات الدقيقة لتربة غابات الصنوبر في اتروش وزاويته من محافظة دهوك شمال العراق با ستخدام خمسة طرائق عزل للفطريات وذلك لغرض مقارنة تنوع الفطريات الدقيقة لطبقات التربة لكلا الموقعين. تم عزل وتشخيص احدى وخمسون نوعا من الفطريات تعود الى ٢٦ جنسا" فضلا" عن الخمائر والخيوط الفطرية غير المتجرثمة. اظهر الجنس اسبرجلس اعلى تنوع وتمثل بـ ١٧ نوع. كانت الانواع ذات اللون الاسود من الجنس اسبرجلس اكثر شيوعا" وتمثلت بخمسة انواع هي:

A.awamori, A.foetidus, A niger, A. sclerotioniger and *A.vadianes.*

اما الجنس *Penicillium* حل بالمرتبة الثانية بعد الاسبرجلس وتمثل بستة انواع شملت

P. cammeberti, P. citrinum, P. coryophilum, P. digitatum , p. glabrum and *P. roquefotri*

اما الاجناس *Absidia*, *Cladosporium*, *Emericella, Mucor and Paecilomyces*

فتمثلت بنوعين لكل منهما.اما بقية الاجناس و التي شملت

Alternaria, Cunnighamella, Chaetomium, Corynascus, Eupenicillium, Fusarium, Gymnoascus, Melanocarpus, Monilia, Pestatiolopsis, Phaeoacremonium , Phialophora, Rhizopus, Sordaria, Stachybotrys, Scytalidium, Thermomyces, Trichoderma and *Ulocladiu*

فتمثلت بنوع واحد لكل منهما. سجل اعلى معامل تشابه لمجتمع الفطريات المستوطنة للتربة ما بين O1 layer. O2 layer (zawita) and (Atrush) اما اقل معامل تشابه سجل ما بين طبقة(zawita) O2 مع O2 (Atrush). سجل النوعان *Absidia spinosa,Mucor pleumbeus* لاول مرة في العراق.

RESISTANCE OF *PSEUDOMONAS AERUGINOSA* FROM CLINICAL AND ENVIRONMENTAL SOURCES TO HEAVY METALS IN HILLA CITY, IRAQ

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Abstract

The study included 300 samples collected from 150 clinical and 150 hospital environmental sources. Only 43 (14.3%) isolates belonged to *Ps. aeruginosa*. The isolates were tested for their susceptibility to 7 types of heavy metals (HM) namely Copper Sulfate, Silver Sulfate, Mercury chloride, Lead nitrate, Zinc sulfate, Cadmium sulfate, and Nickel sulfate. The screening test for ability of the isolates to resist HM was detected using lead nitrate in concentration of 400µg/ml. Results revealed that 37/43 were resistant to lead nitrate (400µg/ml). The MIC of 7 HM was detected by agar dilution and pouring method. Results revealed that most of the isolates were resistant to 7 HM in some of concentrations. The plasmid content was investigated for all 37 isolates of *Ps. aeruginosa* (34 clinical and 3 environmental). Results revealed that most isolates 32/37 harbored large (mega) plasmid. Biofilm production of *Ps. aeruginosa* isolates was investigated; results showed that 20/43 (47%) of isolates had biofilm. This study concluded that the increase of HM resistance was correlated with biofilm production for some HM used. The bacterial curing is proceeding for one isolate of *Ps. aeruginosa* (*Ps.*3). The results showed survived resistance to all HM used, which may be due to HM resistance trait was carried out on bacterial chromosome rather than plasmid.

Keywords: *Pseudomonas Aeruginosa, Resistance, Heavy Metals, Environmental.*

Introduction

Heavy metals, particularly silver and mercury, have a variety of applications in controlling microbial population (Kenneth and Jeffery, 2006). Silver salts alone or in combination with other drugs appear to have a significant potential as topical antimicrobial agent (Fox et al., 1977; De Gracia, 2001). Mercury in the form of less toxic organic compounds is being used as skin disinfectant (Gerald, 2007). Copper is considered as a safe agent to humans, as demonstrated by the widespread and prolonged use by women of copper intrauterine devices (Anonymous, 2002; O'Brien et al., 2008).

Pseudomonas aeruginosa is pathogen that causes a substantial portion of hospital infections. It is frequently multi drug resistant, which contributes to the high morbidity and mortality of patients in intensive care units (ICUs), burn units and surgery wards. A major reason for its prominence as a pathogen is its high intrinsic resistance to antibiotics and heavy metals (Fluit et al., 2000). This a ubiquitous, environmentally important microbe that may employ many resistance mechanisms against different heavy metals, such as the *mer* operon that reduces toxic Hg^{2+} to volatile Hg^{0}, which then diffuses out of the cell (Outten et al., 2000).

Pseudomonas aeruginosa is a prevalent hospital pathogen that is well known for its ability to form biofilms that are recalcitrant to many different antimicrobial treatments (Harrison et al., 2008).

It was found that biofilms were from 2 to 600 times more resistant to heavy metals stress than free-swimming cells. They also showed that biofilms are more resistant to heavy metals than either stationary-phase or logarithmically growing plankotonic cells (Teitzel and Parsek, 2003).

The aim of this study was to detect the prevalence of heavy metals resistant (HMR) *Ps. aeruginosa* isolates recovered from clinical and environmental samples and studying the correlation between biofilm production and HM resistance**.**

Materials and Methods

This study included 300 samples (150 Clinical and 150 Environmental samples). The clinical specimens were collected from patients suffering from burns, wounds, and otitis media, who attending Hilla teaching hospital; whereas hospital environmental samples included catheters, beds, bath rooms, fomites, wall of wards, and some types of disinfectants at the same hospital.

All samples were inoculated on MacConkey agar, Nutrient agar, and selective medium (cetrimide agar), then incubated at 37ºC for 24-48 hrs. *Ps. aeruginosa* were identified by routine diagnostic tests including cellular, cultural and biochemical characteristics.

All isolates were subjected to susceptibility testing by screening test using agar medium supplemented with ($PbNO_3$ 400µg/ml). The isolates were also tested for their susceptibility to 7 heavy metals (HM) represented by; Copper Sulfate, Silver Sulfate, Mercury chloride, Lead nitrate, Zinc sulfate, Cadmium sulfate, and Nickel sulfate. The minimum inhibitory concentration (MIC) of 7 HM was detected by agar dilution method (silver, zinc, cadmium, nickel) and pouring method (lead, copper, mercury), based on standard methods (Riley and Mee, 1982; Forbes, 1998).

The following concentrations of heavy metals were prepared:

1- 0.0001M, 0,001M, 0.01M for Cadmium and 0.0001M, 0.001M, 0.01M, 0.1M for Nickel, Zinc, and Silver.

2- (100, 200, 400, 800, 1600, 2400, 3200 µg/ml) for lead; (100, 200, 400, 800, 1600, 1750, 3200 µg/ml) for Copper; and (2.7, 5.4, 10.8, 21.6, 43.2, 54.3, 86.4 µg/ml) for Mercury.

Biofilm production, Plasmid profile, and Plasmid curing were studied as follows:

Biofilm formation was determined using tissue culture-treated, 96-well polystyrene plates, based on the methods of Christensen et al (1985) and Ziebuhr et al (1997).

Plasmid DNA extraction of gram negative bacteria was performed using Geneaid kit, the steps of the method was according to the manufacturing company (Geneaid kit, USA) and plasmid profile was carried out by electrophoresis (Sambrook and Rusell, 2001). Plasmid curing was carried out using Elevated Temperature method according to Kheder (2002).

Results and Discussion

Out of the 300 samples, only 43 (14.3%) isolates belonged to *Ps. aeruginosa*. 40 (26.6%) of these isolates belonged to clinical samples and 3 (2%) isolates belonged to the *Ps. aeruginosa*.

Heavy metals resistance and MIC of isolates:

All isolates were subjected to susceptibility testing by screening test using agar medium supplemented with $PbNO_3$ 400µg/ml. Results revealed that 37 isolates (85%) were resistant to lead nitrate, these isolates were distributed into 34 clinical and 3 environmental samples (Table 1).

Vaca Pacheco *et al*. (1995) used lead nitrate as a screening test for detection of heavy metals resistance in *P. aeruginosa*, and they found all their isolates were resistant to lead nitrate ($PbNO_3$) at a concentration of 400µg/ml.

Table (1) Numbers and percentage of clinical and environmental isolates of *Pseudomonas aeruginosa* detected by screening test:

Susceptibility to $PbNO_3$ (400µg/ml)	No. of isolates (%)		Total	(%)
	Clinical	Environmental		
Resistant	34(85%)	3(100%)	37	85%
Sensitive	6 (15%)	0	6	15%
Total	40(92.8%)	3(6.9%)	43	100%

Bacterial resistance to heavy metals (Table 2, 3) shows the MIC of *Ps. aeruginosa* to all studied heavy metals. In case of silver sulfate ($AgSO_4$), results showed that 34:37 isolates were resistant to $AgSO_4$ in concentration 0.0001M and the MIC of all isolates was 0.01M.

In case of zinc sulfate ($ZnSO_4$), four isolates were sensitive to $ZnSO_4$ in low concentration 0.0001M. The MIC of all isolates was 0.1M. In case of cadmium sulfate ($CdSO_4$), five isolates were sensitive to $CdSO_4$ in low concentration 0.0001M. The MIC values of all isolates were 0.1M. In case of nickel sulfate ($NiSO_4$), the results indicated the all of isolates were resistant to $CdSO_4$ in concentration 0.0001M and 0.001M. The MIC of all isolates was 0.01M (Table 2).

In case of copper sulfate ($CuSO_4$), results indicated that all isolates were resistant in concentrations of 100, and 200µg/ml and the MIC of most of the isolates was 1600 µg/ml. (Table 3).

In case of mercury chloride ($HgCl_2$), two isolates were sensitive to $HgCl_2$ in low concentration 2.7 µg/ml. The MIC of all isolates was 86.4µg/ml.

In case of lead nitrate ($PbNO_3$), results revealed that 30:37 isolates were resistant in

concentration of 2400 µg/ml and the MIC of all isolates was 3200 µg/ml.

Table (2): MIC values of *Pseudomonas aeruginosa* isolates to silver sulfate, zinc sulfate, cadmium sulfate, nickel sulfate in molar concentrations.

Isolates	MIC of Silver sulfate	MIC of Zinc sulfate	MIC of Cadmium sulfate	MIC of Nickel sulfate
*Ps.*1	0.01	0.1	0.001	0.01
*Ps.*2	0.01	0.1	0.001	0.01
*Ps.*3	0.01	0.1	0.01	0.01
*Ps.*4	0.01	0.1	0.001	0.01
*Ps.*5	0.01	0.1	0.001	0.01
*Ps.*6	0.01	0.1	0.01	0.01
*Ps.*8	0.01	0.01	0.01	0.01
*Ps.*9	0.01	0.1	0.01	0.01
*Ps.*12	0.01	0.1	0.001	0.01
*Ps.*13	0.01	0.1	0.001	0.01
*Ps.*14	0.01	0.1	0.001	0.01
*Ps.*15	0.01	0.1	0.01	0.01
*Ps.*16	0.001	0.01	0.001	0.01
*Ps.*17	0.001	0.1	0.0001	0.01
*Ps.*19	0.0001	0.0001	0.001	0.01
*Ps.*20	0.001	0.01	0.001	0.01
*Ps.*21	0.001	0.01	0.001	0.01
*Ps.*22	0.01	0.01	0.001	0.01
*Ps.*24	0.001	0.01	0.001	0.01
*Ps.*25	0.01	0.01	0.001	0.01
*Ps.*26	0.001	0.01	0.001	0.01
*Ps.*27	0.01	0.01	0.01	0.01
*Ps.*28	0.001	0.1	0.01	0.01
*Ps.*29	0.001	0.01	0.001	0.01
*Ps.*30	0.001	0.01	0.001	0.01
*Ps.*31	0.0001	0.0001	0.01	0.01
*Ps.*32	0.001	0.1	0.01	0.01
*Ps.*33	0.001	0.01	0.01	0.01
*Ps.*34	0.001	0.01	0.001	0.01
*Ps.*36	0.0001	0.0001	0.01	0.01
*Ps.*37	0.001	0.01	0.0001	0.01
*Ps.*38	0.001	0.01	0.01	0.01
*Ps.*39	0.001	0.01	0.001	0.01
*Ps.*40	0.01	0.01	0.0001	0.01
*Ps.*41	0.001	0.01	0.01	0.01
*Ps.*42	0.001	0.1	0.0001	0.01
*Ps.*43	0.001	0.0001	0.0001	0.01

The interpretation of these results may be due to the fact that *Ps. aeruginosa* has many mechanisms for heavy metals resistance; firstly, the accumulation of specific ions can be diminished, not by interference with uptake but by active extrusion of the heavy metals ion from the cells. This mechanism is specific only for *Pseudomonas* spp. Secondly; cations can be segregated into complex compound by thiol-containing molecules and then ejected from the cell. Thirdly, some metal ions may be reduced to a less toxic oxidative state by the complex enzymes and special oxidation mechanisms in the cells and finally, for many metals resistance and homoeostasis where is a combination of two or three of the mentioned basic mechanisms that is the case which *Ps. aeruginosa* success (Abdul-Sada, 2008).

Prasad *et al.* (2009) found that all isolates were sensitive to heavy metals (Cd^{2+}, Ag^{+}, Ar^{2+}, Co^{2+}, Ni^{2+}, Hg^{2+}, and Pb^{2+}) at a concentration of 0.1M, and most of them were resistant to heavy metals at a concentration of 0.0001M. Singh *et al* (2010) found that the MIC values of Ni^{2+} were ranged from 80-250 µg/ml, Cd^{2+} was (80-210 µg/ml).

The results of this study are similar to those obtained by Prasad *et al* (2009) who found that all isolates of *P. aeruginosa* were resistant to silver nitrate at a concentration of 0.0001M and all isolates were sensitive to silver nitrate at concentrations of 0.01M and 0.1M. Dong *et al*.

(2001) found 100 µg/ml of sliver nitrate is effective to prevent *P. aeruginosa* ATCC 27853 production biofilm and inhibition of bacterial attachment.

In a local study, Abdul-Sada (2008) found that *P. aeruginosa* isolated from wastewater in Basrah, Iraq, were resistant to Zn_2O_3 and cadmium chloride at concentrations of 0.4M, and 0.1M, respectively, while Xiao-xi *et al*. (2009) found that *P. aeruginosa* isolate E_1 was resistant to Zn^{2+} and Cd^{2+} in concentrations of 16.5 mmol/L (0.0165M) and 18.5 mmol/L (0.018M) respectively. Nies (2003) interpreted that *P. aeruginosa* respond to excess Zn^{2+} and cd^{2+} by metal-inducible resistance mechanisms, Zn^{2+} and Cd^{2+} resistance in bacteria is mainly based on active efflux of metal ions to prevent toxic effects in the cell.

Regarding to mercury chloride, karbasizaed *et al* (2003) revealed that coliforms were tolerant to mercury chloride was in 54.3 µg/ml, while Prasad *et al* (2009) found that all isolates of *P. aeruginosa* were sensitive to mercury chloride in concentration 0.0001M, 0.001M , 0.01M and 0.1M. *Pseudomonas aeruginosa* were able to resist to mercury because it has *mer operon* that reduced toxic Hg^{2+} to volatile Hg^0, which then diffuses out of the cell.

According to lead nitrate, the results showed that all isolates were resistant to lead nitrate and the MIC values ranged from 800-3200 µg/ml (Table 3). These results are similar to that obtained by Karbasized *et al* (2003) who revealed the coliforms were tolerant to lead nitrate was in a MIC of 3200 µg/ml. Xiao-xi *et al*. (2009) found *P. aeruginosa* isolate E_1 was resistant to pb^{+2} in concentration 10.0 mmol/L (0.01M). Prasad *et al*. (2009) found that all isolates of *P. aeruginosa* were sensitive to lead nitrate at concentrations of 0.001M, 0.01M and 0.1M. Many authors found that *P. aeruginosa* were resistant pb^{2+} by the system localized in cad AC operon, cad A catalyzed the active efflux of Cd^{2+}, Zn^{2+}, and Pb^{2+}, also they found Pseudomonads have P-type ATPase that can resist Pb^{2+} (Nucifora *et al*., 1989).

Table (3): MIC values of *Pseudomonas aeruginosa* isolates to copper sulfate, mercury chloride, and lead nitrate in (µg/ml) concentrations.

Isolates	MIC of Copper sulfate	MIC of Mercury chloride	MIC of Lead nitrate
*Ps.*1	1600	2.7	3200
*Ps.*2	1750	54.3	3200
*Ps.*3	1750	86.4	3200
*Ps.*4	1600	86.4	3200
*Ps.*5	1750	43.2	3200
*Ps.*6	1750	54.3	3200
*Ps.*8	1750	86.4	3200
*Ps.*9	1750	86.4	3200
*Ps.*12	1600	54.3	3200
*Ps.*13	1750	43.2	3200
*Ps.*14	1750	86.4	800
*Ps.*15	1600	86.4	3200
*Ps.*16	1600	86.4	3200
*Ps.*17	1600	54.3	3200
*Ps.*19	1600	86.4	3200
*Ps.*20	3200	86.4	3200
*Ps.*21	1600	86.4	800
*Ps.*22	1600	2.7	2400
*Ps.*24	400	54.3	3200
*Ps.*25	1600	43.2	3200
*Ps.*26	1600	86.4	3200
*Ps.*27	1600	21.6	2400
*Ps.*28	800	43.2	2400
*Ps.*29	400	21.6	3200
*Ps.*30	1600	21.6	3200
*Ps.*31	1600	21.6	1600
*Ps.*32	1600	21.6	3200
*Ps.*33	1600	21.6	3200
*Ps.*34	1600	21.6	3200
*Ps.*36	1600	86.4	3200

*Ps.*37	800	21.6	3200
*Ps.*38	3200	21.6	3200
*Ps.*39	1600	43.2	١٦٠٠
*Ps.*40	1600	86.4	3200
*Ps.*41	1600	54.3	3200
*Ps.*42	1600	54.3	3200
*Ps.*43	1600	43.2	3200

Biofilm formation:

The biofilm formation by *Ps. aeruginosa* isolates was investigated. The results showed that 20/43 (47%) of isolates had biofilm (Table 4). Results also showed that all environmental isolates (3 isolates) and 17/40 (42.5%) of clinical isolates were biofilm producers. The relationship between biofilm production and heavy metal resistance (HMR) was studied. It was found that the HMR of *Ps. aeruginosa* isolates is not correlated with production of the biofilm. From these results, this study concluded that the increase of HM resistance was correlated with biofilm production for some (but not all) HM used.

Table (4): Biofilm production by *Pseudomonas aeruginosa* isolates recovered from clinical and environment samples

Isolate No.	A 492 (≥ 0.17)*	Biofilm production
Ps. 1	0.11	-
Ps. 2	0.20	+
Ps. 3	0.23	+
Ps. 4	0.17	+
Ps. 5	0.09	-
Ps. 6	0.11	-
Ps. 7	0.22	+
Ps.8	0.12	-
Ps. 9	0.22	+
Ps. 10	0.21	+
Ps.11	0.14	-
Ps. 12	0.11	-
Ps. 13	0.14	-
Ps. 14	0.19	+
Ps. 15	0.10	-
Ps. 16	0.15	-
Ps. 17	0.24	+
Ps. 18	0.14	-
Ps.19	0.11	-
Ps.20	0.20	+
Ps.21	0.16	-

No. of isolates	**A 492 (≥ 0.17)***	**Biofilm production**
Ps.22	0.09	-
Ps.23	0.09	-
Ps.24	0.17	+
Ps.25	0.20	+
Ps.26	0.26	+
Ps. 27	0.11	-
Ps.28	0.30	+
Ps.29	0.11	-
Ps.30	0.11	-
Ps. 31	0.16	-
Ps. 32	0.11	-
Ps. 33	0.12	-
Ps.34	0.20	+
Ps.35	0.14	-
Ps. 36	0.16	-
Ps. 37	0.29	+
Ps.38	0.24	+
Ps. 39	0.20	+
Ps.40	0.25	+
Ps.41	0.24	+
Ps.42	0.12	-
Ps.43	0.83	+

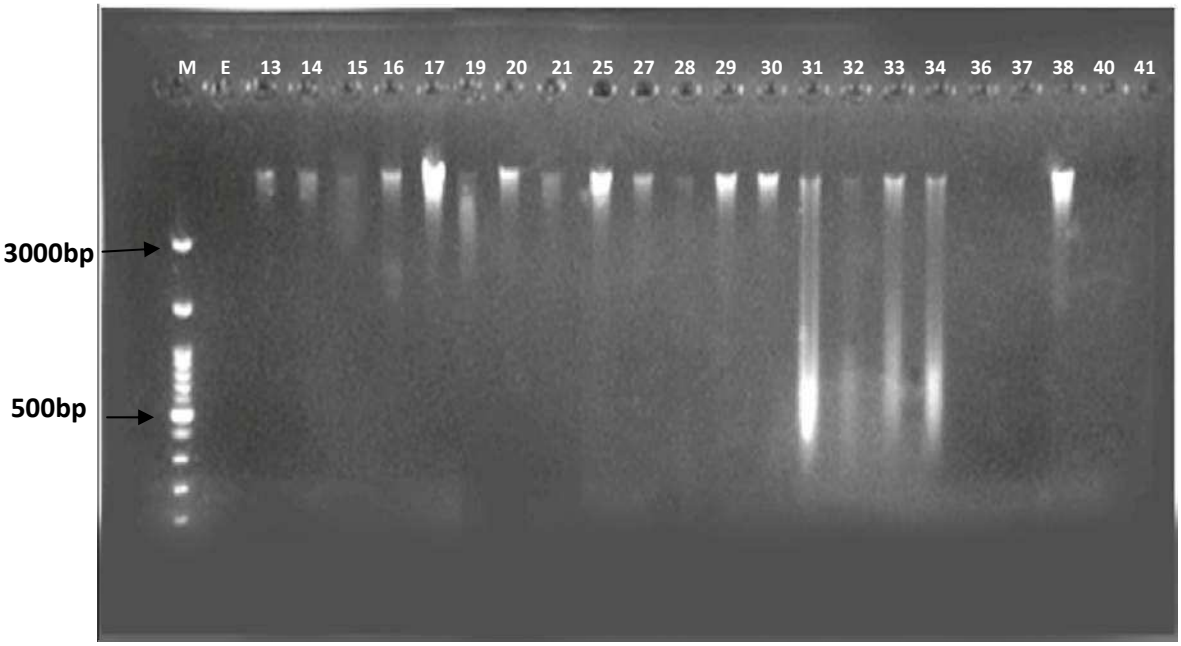
* The number between brackets indicates the standard value of biofilm production by ELIZA technique.

Plasmid profile and Curing of bacterial plasmids:

The plasmid content was investigated for all 37 isolates of *Ps. aeruginosa* (34 clinical and 3 environmental) (Figure 1). Results revealed that most isolates 32:37 harbored large (mega) plasmid with large (huge) molecular weight that couldn't be detected using 3000 bp size marker (ladder).

Many researchers worldwide reported that HMR in *P. aeruginosa* is carried on large (mega) plasmids. Raja and Selvam (2009) revealed isolate *P. aeruginosa* exhibited resistance to heavy metals such as cadmium, chromium, nickel and lead, due to the presence of plasmid DNA, which was designated as pBC15. The size of this plasmid DNA was approximately 23 kb, and they suggested that nickel and ampicillin resistance gene was conferred by plasmid DNA. Nikbin *et al* (2007) revealed that *P. aeruginosa* isolated from hospital in Tahran, Iran has plasmid with molecular weight 100 kbp. However in this study a large size marker (40000bp) was not available at the period of the study, so only 3000bp marker was used and all of the plasmid bands (in the gel) were out of ladder.

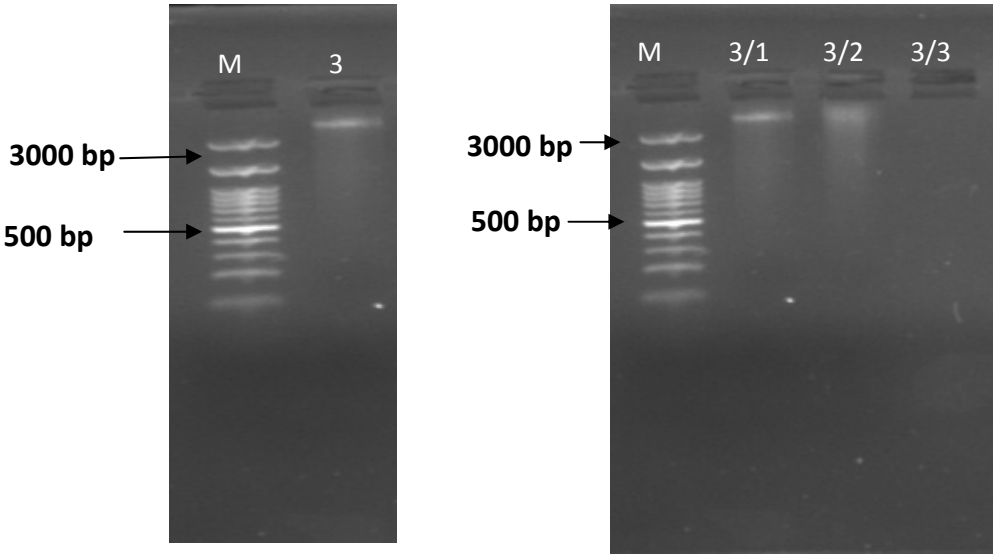
Figure (1): Gel electrophoresis of plasmid DNA content of *Ps. aeruginosa* isolates after (1:30) hr. at (60) voltage.



Lane (M): DNA molecular size marker (3000-bp ladder).
Lane (E): Show negative control (*E. coli standard strain MM294).*
Lanes: (P13), (P14), (P15), (P16), (P17), (P19), (P20), (P21), (P25), (P27), (P28), (P29), (P30), (P31), (P32), (P33), (P34), (P36), (P37), and (P38) shows clinical isolates.
Lanes :(P40), (P41) Show environmental isolates.

The bacterial curing was concluded for one isolate *Ps. aeruginosa* (*Ps.*3) (Figure 2). The results showed survived resistance to all HM. This result indicates that the HM resistance trait was carried on chromosome rather than plasmid. This could be due to that the plasmid is not cured out because it is really difficult to cure large mega plasmids. Many isolates of *P. aeruginosa* have no plasmid content and still show heavy metals resistance that lead to think that gene responsible for these resistances found on the chromosome (Raja and Selvam, 2009).

Figure (2): Gel electrophoresis of plasmid DNA content of *Ps. aeruginosa* isolate before and after curing after (1:30) hr. at(60) voltage.



Before curing **After curing**

Lane (M): DNA molecular size marker (3000-bp ladder).
Lane ($P_{3/1)}$: shows clinical isolate (first dilution).
Lanes ($P_{3/2}$): shows clinical isolate (second dilution).
Lanes ($P_{3/3}$): shows clinical isolate (third and last dilution).

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مقاومة الزوائف الزنجارية المعزولة من مصادر سريرية وبيئية للمعادن الثقيلة في مدينة الحلة، العراق

الملخص

تضمنت هذه الدراسة جمع ٣٠٠ عينة، ١٥٠ سريرية و ١٥٠ جمعت من بيئة المستشفى لغرض عزل بكتريا الزوائف الزنجارية. تم عزل ٤٣ (١٤,٣%) عزلة من الزوائف الزنجارية. تم إجراء مسح أولي للكشف عن العزلات المقاومة للمعادن باستخدام نترات الرصاص بتركيز (400 µg/ml) وأظهرت النتائج أن 37:43 من العزلات كانت مقاومة لهذا التركيز. تم الكشف عن حساسية هذه العزلات المقاومة لعدد من المعادن الثقيلة (كبريتات الفضة، كبريتات الزنك، كبريتات الكادميوم، كبريتات النيكل، كبريتات النحاس، كلوريدات الرئبق، ونترات الرصاص) من خلال تحديد التركيز المثبط الأدنى لهذه المعادن وبطريقتي التخفيف بالأكار وطريقة صب الأطباق وأظهرت النتائج ان معظم العزلات كانت مقاومة لهذه المعادن في بعض التراكيز. تم الكشف عن المحتوى البلازميدي للعزلات المقاومة للمعادن الثقيلة وأظهرت النتائج أن معظم العزلات ٣٢:٣٧ أحتوت على بلازميد عملاق. تم التحري عن انتاج الغشاء الحيوي للعزلات ووجد ان (٤٧%) من العزلات منتجة له. وأظهرت النتائج ان زيادة المقاومة للمعادن الثقيلة كان مرتبطا مع قابلية بعض العزلات على انتاج الغشاء الحيوي. أظهرت نتائج تحييد البلازميد البكتيري للعزلات بقاء المقاومة للمعادن الثقيلة بعد التحييد مما يشير الى ان صفة مقاومة المعادن الثقيلة محمولة على الكروموسوم وليست على البلازميد البكتيري.

HEPATITIS C VIRUS PREVALENCE IN HAEMODIALYSIS PATIENTS FROM THREE CENTERS IN BAGHDAD, IRAQ: A SURVEY BY POLYMERASE CHAIN REACTION AND SEROLOGICAL METHODS

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Abstract:

Hepatitis C virus infection is a major health problem among haemodialysis patients in developing countries. Nosocomial transmission of HCV infection was a considerable route, particularly during the outbreaks of infection. To compare serological and molecular methods for detection of HCV infection serum samples were screened for anti-HCV antibodies using a fourth generation enzyme-linked immunosorbent assay (ELISA) and positive samples were confirmed by immunoblot assay. All seropositive and seronegative samples were screened for the presence of HCV-RNA by using reverse transcriptase PCR (RT-PCR). The overall prevalence was (41.10%) in the three centers (range: 26.05% to 62.82%) with higher prevalence in Al-Kadhimiya Teaching Hospital. All seropositive samples were tested by reverse transcriptase PCR, and 24/92 (26.09%) of confirmed samples were found to contain HCV-RNA. Additionally, 2/5 (40%) of immunoblot-indeterminate and 1/3 (33.33%) of immunoblot-negative samples were also found to be HCV-RNA positive. Also all seronegative samples were screened for the presence of HCV-RNA by using pooling strategy and 2/136 (1.47%) of anti-HCV negative samples were found to be HCV-RNA positive. Our data emphasize the need for stricter adherence to infection control measures in haemodialysis centers and reinforce the importance of screening by both PCR and serological methods at regular intervals to identify all HCV-infected patients.

Key words: *HCV, Haemodialysis, Serological test, RT-PCR.*

Introduction:

Hepatitis C virus (HCV) is blood-borne pathogen that appears to be endemic in most parts of the world. It is estimated that there are 170 million HCV-infected persons worldwide (Al Dhahry *et al.*, 2003; and Senevirathna *et al.*, 2008).The populations most affected by HCV are patients that undergo multiple blood transfusions, individuals who are intravenous and inhalant drug users, hemophiliacs, and haemodialysis patients (Alavian *et al.,* 2011). HCV infection is a major health problem among dialysis patients in the developing countries. The higher prevalence in developing countries in comparison with developed countries reflects many factors including socioeconomic factors, bad infection control measures, use of blood transfusion before the routine use of erythropoietin to treat anemia and the higher prevalence of HCV among general population in developing countries (Chong and Zinna, 2008). Currently there are no vaccine and post exposure anti-viral prophylaxis to prevent HCV infection. Therefore, identification of HCV infected in haemodialysis (HD) centers can reduce the risk of nosocomial transmission of HCV and its clinical complication (Samimi-rad *et al.*, 2008). In Iraq, the prevalence of anti-HCV was reported between (40.2%) in renal dialysis unit patients in Mosul (Mohammed, 1997) to (62%) in haemodialysis patients (Khalaf *et al.*, 1996). The prevalence rates reported in haemodialysis patients in Middle Eastern countries are 68% in Saudi Arabia with a range of 14.5% to 94.7%, 26% in Oman, and 80% in Egypt. The seroprevalence of HCV in haemodialysis centers in Jordan was 34.6% compares with 45% in Tunisia and 45% in Syria (Bdour, 2002). In Turkey, the prevalence of HCV infection among HD has been reported between 31.4% and 51% (Kaya, 2008). In Iran, several studies were done regarding HCV seroprevalence among HD patients (Ansar and Kooloobandi, 2002; Alavian *et al.*, 2003; Amiri *et al.*, 2005; and Hosseini-Moghaddam *et al.*, 2006), in the largest study done by Hosseini-Moghaddam and his colleagues in 2006, 45 HD centers were enrolled in the study, including 1914 HD patients, HCV was found in 8.1% of studied population (Hosseini-Moghaddam *et al.*, 2006). The prevalence rates reported are 1%–29% from Western Europe, 8%–36% from North America, 5.9% in Australia, and 44%–60% in Far Eastern countries (Hayat *et al.*, 2010).

Subjects, Materials and Methods:

Subjects: A total of 236 dialysis patients, 150 (63.6%) male and 86 (36.4%) female, their age ranged from 15 to 78 (44.39 ± 15.06 S.D.) years. They were attendants the three HD centers in Baghdad; Al-Yarmouk Teaching Hospital, Al-Kadhimiya Teaching Hospital and Al-Karama Hospital. Samples were taken between May and October 2010. The mean duration of HD treatment was (33.91 ± 25.75) months. All patients were dialyzed 2 or 3 times per week and each HD treatment took three to four hours, the patients were distributed into two shifts depending on their haemodialysis centers. Dialyzer membranes were disposable and single use. The clinical diagnosis was obtained from patient records and interview and ethical approval for use of all specimens was obtained. Our exclusion criteria were peritoneal dialysis or history of receiving antiviral and/or interferon therapy for HCV (+) subjects.

Samples: Blood was obtained by vein puncture immediately before HD sessions. Sera were separated from whole blood under optimal conditions for RNA extraction. For this purpose, the blood samples were allowed to clot in the room temperature for 20 minutes and then centrifuged at 2,000 rpm for 10 minutes (-4ºC). All samples were divided into three aliquots then immediately frozen and stored at (-20ºC) and (-80ºC), for serological and molecular assays respectively to minimize degradation of viral nucleic acid, prevent cross contamination and unnecessary thawing and freezing.

Materials and Methods: For anti-HCV antibodies detection two commercial kits were utilized in this part of study. The initial screening for anti-HCV IgG antibody was determined by fourth generation enzyme-linked immunoassay (ELISA) (Bioelisa HCV 4.0 ELISA, Biokit, Spain). The results were inter-preted according to manufacturers' instructions. All ELISA positive samples were subjected to confirmatory test using immunoblot assay (EIBA) (Bioblot HCV, Biokit Spain). All seropositive samples were tested individually for the presence of HCV RNA by qualitative RT-PCR (Sacace Biotechnologies, REF V-1-100R, Italy). To permit the molecular analysis of the large number of seronegative samples, a pooling strategy was developed, similar to the method described by Schneeberger *et al.* (1998). This involved the pooling of four seronegative serum samples and the analysis of the mixture for the presence of HCV RNA. Twenty-five µl of each of the four samples were mixed together, and then 100µl pool was used for the assay. RT-PCR based on four major processes: isolation of HCV RNA from specimens, reverse transcription of the RNA, nucleic acid amplification and detection of the amplified products on agarose gel. To avoid possible contamination with exogenous sequences during extraction or amplification, all nucleic acid extraction, amplification, and detection steps were performed in separate laboratories. Negative and positive controls were extracted, reverse transcribed, and amplified in each batch of samples tested by PCR. All amplified product were analyzed using electrophoresis.

Statistical analysis: Descriptive analysis was done using the statistical package for social studies (SPSS) program for windows software package release 15.

Results:

Seroprevalence of HCV in haemodialysis patients:

The collected sera were subjected to serological screening and confirmation analysis to determine the presence of HCV. The results are summarized in Table (1). Sera from 100/236 (42.37%) patients were found to be anti-HCV positive by ELISA and 97 (41.10%) were subsequently confirmed as being positive by bioblot; 92 (38.98%) of these sera were anti-HCV bioblot positive giving the true seroprevalence of anti-HCV antibody, five sera gave indeterminate results on immunoblotting and three were negative.

Table (1): Anti-HCV seropositivity by ELISA and bioblot in 236 HD patients.

Characteristic of anti-HCV	No.	%
Total H.D. patients tested	236	100
Non reactive in ELISA	136	57.63
Reactive in ELISA	100	42.37
Bioblot positive	92	38.98
Bioblot indeterminate	5	----
Bioblot negative	3	----

Variation in the prevalence of anti-HCV antibody was observed between the haemodialysis centers. The distribution of HCV-infected patients among the three dialysis centers is shown in Figure (1). Anti-HCV antibody was found in 31/119 (26.05%), 49/78 (62.82%), 17/39 (43.58%) in Al-Yarmouk Teaching Hospital, Al-Kadhimiya Teaching Hospital and Al-Karama hospital, respectively. Thus, the prevalence of HCV infection ranged from 26.05% to 62.82%.

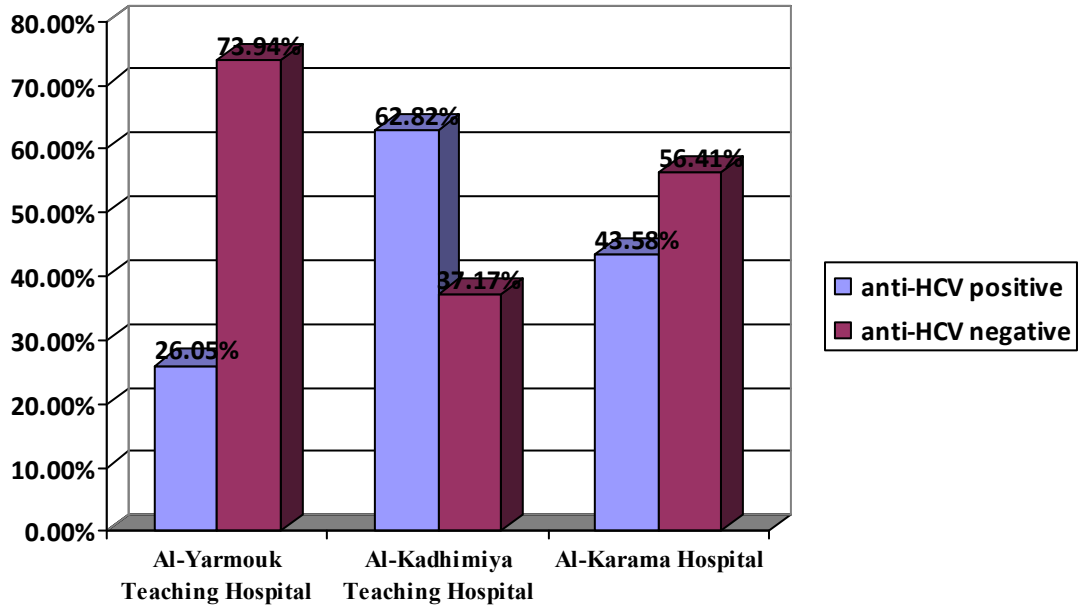

Figure (1): Seroprevalence of HCV infection in the three hemodialysis centers.

HCV RNA prevalence in haemodialysis patients:

HCV-RNA was detected in 29/236 (12.29%) of haemodialysis patients sera. In 92 of 100 (92%) confirmed anti-HCV antibody by bioblot; 24 of these sera were HCV RNA-positive. Five sera gave indeterminate results on immunoblotting; two were HCV RNA-positive and three were negative on immunoblotting; one was HCV RNA-positive), as shown in Table (2) and Figure (2).

All 136 anti-HCV antibody-negative sera were tested by RT-PCR divided among 34 pools. Among the 34 pools of seronegative samples, 2 yielded positive signals for HCV RNA. Then these two positive pools were retested individually by RT-PCR and resulted in two of 136 (1.47%) seronegative sera were confirmed to be HCV-RNA positive. The combination of serological and molecular methods resulted in the most accurate estimation of the number of HCV infections among haemodialysis patients. Using only antibody assays in this population, three HCV RNA-positive patients would have been missed.

Table (2): Comparison of ELISA, bioblot and RT-PCR results: data on anti- HCV positive and negative HD patients with HCV RNA detection.

Test results		No. of patients (%)			
EIA	**Bioblot**	**Total**	**%**	**PCR positive**	**%**
Positive	**Positive**	92/100	92%	24/92	26.09%
Positive	**Indeterminate**	5/100	5%	2/5	40%
Positive	**Negative**	3/100	3%	1/3	33.33%
Negative		136/236	57.63%	2/136	1.47%
Total		236	100%	29/236	12.29%

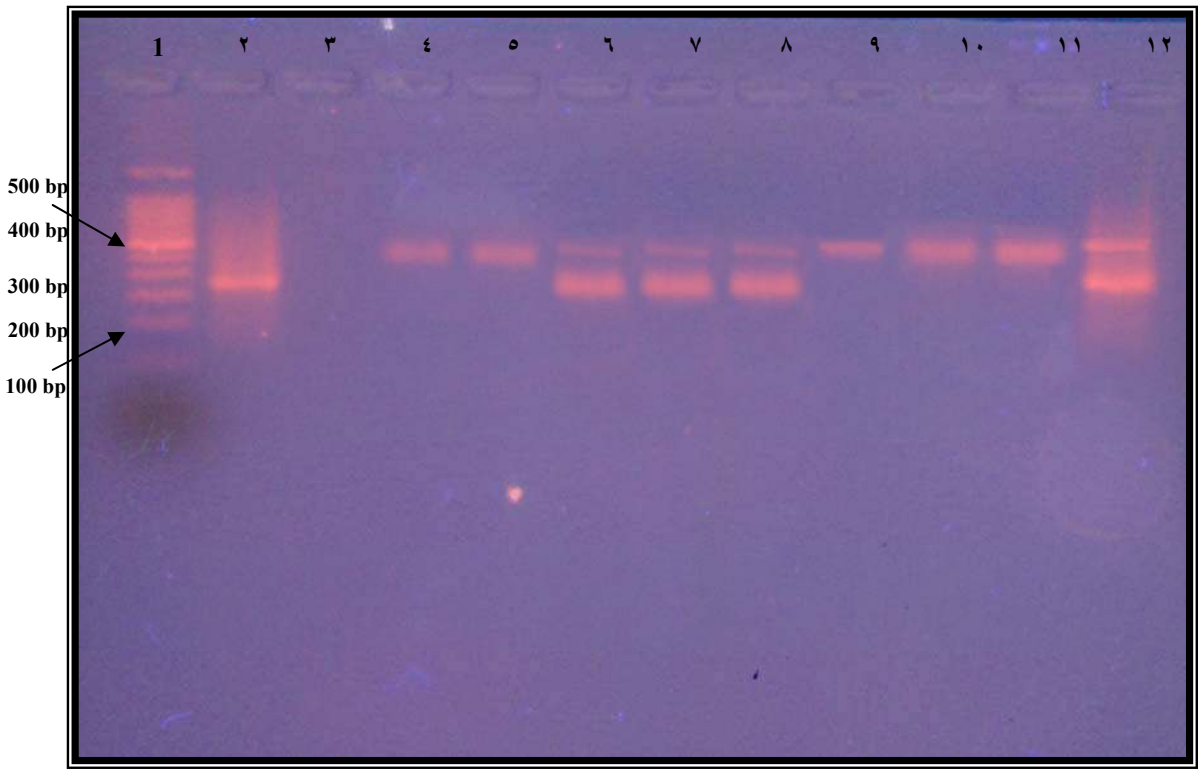

Figure (2): PCR products from haemodialysis patients. Lane 1, DNA marker (100-bp ladder).The 500bp band was present at triple the intensity of the other fragments and serves as a reference indicator, while all other fragments appear with equal intensity on the gel; lane 2, HCV cDNA (C+) serves as positive control for amplification; lane 3, DNA-buffer (C-) serves as negative control for amplification; lane 4, HCV IC Rec Fag serves as internal control; lane 5, Negative control sample (NCS) serves as negative control for RNA isolation; lane 6 to 8, DNA from haemodialysis patient positive for HCV antibodies; lane 9 to 11, DNA from haemodialysis patients negative for HCV antibodies; lane 12, HCVC+ Rec Fag serves as positive control for RNA isolation.

Discussion:

Prevalence of anti-HCV antibody:

Several prevalence studies of HCV infection have been undertaken in HD patients. The prevalence of anti-HCV antibody in HD subjects ranged between 10% and 55% (Horoz *et al.,* 2006). Epidemiological studies about HCV infection among HD patients in Iraq have reported a prevalence of 7.1%-62% in different cities (Khalaf *et al,* 1996; and Kattab; 2008). While the reported prevalence in the general population in Iraq is ranges from (0.2%) to (0.5%) (Fayadh and Jureidini, 2001).

The prevalence of anti-HCV antibody in our study was found to be (41.10%) and ranged from (26.05%) to (62.82%) according to the dialysis center (Table: 1) and (Figure: 1), these results in accordance with that reported by others (Khalaf *et al,* 1996; Mohammed, 1997; Othman and Monem, 2001; and Al-Shohaib *et al.*, 2003). HCV prevalence in haemodialysis patients is highly variable between different countries and between different centers in the same locality (Devesa *et al.*, 1997). This difference can be attributed to several influencing factors. First, these studies were performed in different regions, with various HCV prevalence among their normal population. Second, adherence of haemodialysis centers to precautionary measures might be different between centers of various parts of the same country (Alavian *et al.,* 2011).Technical reasons are likely to account for this difference since a different generation EIA and confirmatory immunoblot assay were used for evaluating HCV-antibody seropositivity.

In this study, different seroprevalence of HCV had detected in the three HD centers in Baghdad. A much higher prevalence (62.82%) of anti-HCV was documented among Al-Kadhimiya Teaching Hospital patients and this strongly points to the magnitude of HCV problem among this selected group of patients. The second high prevalence was found in Al-Karama hospital (43.58%), followed by Al-Yarmouk Teaching hospital which has the lowest prevalence (26.05%) (Figure: 1). Nosocomial transmission of HCV infection has been reported to be a considerable route in modern hospital dialysis centers, particularly during the outbreaks of infection (Sabry *et al.*, 2007; and Arrais *et al.*, 2008).

HCV RNA prevalence in haemodialysis patients:

Detection of HCV-PCR is currently the most sensitive and specific method for detecting active infection and to overcome two other problems: that the serological test can not differentiate between acute and chronic infection and cannot detect evidence of infection during window period (Al-Kubaisy *et al.,* 2003). In the present study RT-PCR was used to screen for the presence of HCV RNA in all 236 serum samples. HCV RNA was detected in 29 (12.29%) samples, Table (2). Of these 236 serum samples, 97 were seropositive and HCV-RNA was detected in 26 (26.80%). We conclude that prevalence of hepatitis C infection is high in HD patients in our region, but not associated with active HCV infection. Similarly, Arababadi *et al*. (2009) from Kerman, Iran found that 30 out of 90 (33.3%) HD patients were RT-PCR positive for HCV-RNA. Also Bdour (2002) from Jordan, found that 30 out of 98 (30.6%) anti-HCV positive sera was harbor HCV-RNA by RT-PCR. In Brazil, HCV viremia was present in 63.5% of the anti HCV positive patients in one study (Carneiro *et al.*, 2001) and (7.6%) in another study (De Albuquergue *et al.*, 2005).

Possible explanations for this low percentage of viremic patients in our population include: the level of viremia may be low and below detectable level of PCR assay at time of sampling, the pattern of fluctuating viremia or intermittent viremia, patients might be cured from HCV infection at time of sampling (Bdour, 2002; Al-Kubaisy *et al.,* 2003; and Wang *et al*., 2004). PCR-based methods reliability may further be compromised if viral RNA is lost in the serum or plasma through storage or improper laboratory handling or if it is absent from the circulation during sample collection (Zein, 2000).

However, it has also been reported that 7–68% of haemodialysis patients have intermittent viremia with periods of undetectable HCV RNA for up to 4 weeks. The viral load is relatively low in this group of patients and long-term maintenance haemodialysis decreases the HCV RNA level but does not produce clearance of viremia (Bdour, 2002; and Horoz *et al.*, 2006). Barril *et al.* (2008) detected the presence of antigenomic HCV-RNA in 53% of HD patients with occult HCV (presence of HCV- RNA in liver in absence of anti-HCV and serum HCV-RNA), showing that HCV is replicating in peripheral blood mononuclear cells (PBMC) and

suggesting that these patients could be potentially infectious; therefore, occult HCV infection could play a role in HCV spread within HD centers which is a very serious problem.

In the present study, PCR was used to screen for the presence of HCV RNA in all 136 seronegative serum samples. HCV RNA was detected in 2 of 136 (1.47%) of the seronegative samples using pooling strategy, retesting positive pools individually confirmed these result. Also HCV RNA was detected in 1 of 3 (33.33%) of the bioblot negative samples which might be bioblot false negative. The presence of HCV viremia in anti-HCV negative haemodialysis patients has been frequently reported by others researchers (Schneeberger *et al.*, 1998; and Carneiro *et al.*, 2001). In contrast, none of the anti-HCV negative patients were shown to be viremic by the PCR as mention by De Albuquergue *et al.* (2005). The detection of HCV RNA in non-hepatitis patients could be explained by the fact that the patients might be in the early stage of acute hepatitis, and the antibody had not been produced yet (Wang *et al.*, 2004). On the other hand, one cannot exclude the existence of impaired immune responses in some of these patients (Schneeberger *et al.*, 1998).

Conclude that the detection of HCV RNA by PCR technique permits direct detection of the presence of the virus and also permits detection of infectivity during the seronegative window which reinforces the importance of screening by both serological and PCR methods at regular intervals to identify all HCV-infected patients.

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انتشار عدوى التهاب الكبد الفايروسي نمط "ج" في مرضى غسيل الكلى من ثلاثة مراكز في بغداد، العراق: دراسة استقصائية بواسطة تفاعل البلمرة والطرق المصلية

الخلاصة:

تعتبر العدوى بالتهاب الكبد الوبائي نمط ج مشكلة صحية كبيرة بين مرضى غسيل الكلى في البلدان النامية. وتعتبر عدوى المستشفيات من اهم طرق انتقال الفايروس، ولا سيما خلال تفشي المرض. للمقارنة بين الطرق المصلية والجزيئية في الكشف عن فيروس التهاب الكبد الوبائي تم فحص العينات المصلية لوجود الاجسام المضادة لفايروس التهاب الكبد باستخدام الجيل الرابع لفحص الامتزاز المناعي المقترن بالأنزيم (ELISA) وتم تأكيد الايجابية باستخدام مقايسة اللطخة المناعية (immunoblot assay). كما وتم اختبار جميع العينات لوجود الحامض النووي الرايبوزي للفايروس (HCV-RNA) بطريقة سلسلة تفاعل البلمرة العكسي (RT-PCR). لقد تم تقييم مدى انتشار العدوى بفايروس التهاب الكبد نمط ج لدى ٢٣٦ من مرضى الغسيل الكلوي في ثلاث من وحدات غسيل الكلى في مدينة بغداد (مستشفى اليرموك التعليمي، مستشفى الكاظمية التعليمي، ومستشفى الكرامة). وكان معدل الانتشار العام (41,10%) في الوحدات الثلاثة (ضمن المجال:٢٦،٠٥ % إلى ٦٢،٨٢%) مع أعلى معدل انتشارسجل في مستشفى الكاظمية التعليمي. وتم اختبار جميع العينات إيجابية المصل بطريقة سلسلة تفاعل البلمره العكسي، وتم العثور على ٩٢/٢٤ (٢٦،٠٩%) من العينات الايجابية باللطخة المناعية أكدت أحتواءها على الحامض النووي الرايبوزي. بالإضافة إلى ذلك تم العثور أيضا على ٥/٢ (٤٠%) من العينات غير محددة باللطخة المناعية ، و ٣/١ (٣٣،٣٣%) من العينات السلبية باللطخة المناعية لتكون ايضاً إيجابية للحامض النووي الرايبوزي. كما تم فحص جميع العينات المصلية السالبة لوجود الاجسام المضادة للفايروس باستخدام استراتيجية التجميع لوجود الحامض النووي الرايبوزي وكانت النتيجة أيجابية في ١٣٦/2 (١،٤٧%). أكدت هذه الدراسة على ضرورة الالتزام الصارم بأجراءات السيطرة على العدوى في مراكز غسيل الكلى وتعزيز أهمية الفحص بواسطة كل من الطرق المصلية و تفاعل البلمره العكسي على فترات منتظمة لتحديد جميع المرضى المصابين بالتهاب الكبد الفايروسي نمط ج.

الكلمات المفتاحية: فايروس التهاب الكبد نمط ج، الغسيل الكلوي، الاختبارات المصلية ، تفاعل البلمره العكسي.

BACTERIOLOGICAL QUALITY OF IMPORTED FROZEN CHICKEN IN SULAIMANI MARKETS

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Abstract

To study the bacteriological quality of imported frozen chicken meat in Sulaimani markets, a total of 360 samples represented whole chickens (160 samples, 80 for each batch), thighs (120 samples, 60 for each batch) and breasts (80 samples, 40 for each batch) belonged to seven commercial trademarks, distributed on two batches, were tested according to international analytical regulations and guides. The mean values of the total plate count (TPC) in batches 1 and 2 of the whole chicken carcasses ranged 0.540-5.720 $\times10^4$ and 0.250-5.720 $\times10^4$ CFU/ g meat respectively, in thighs they ranged 1.170- 17.520 and 0.730- 12.500 $\times10^4$CFU/ g meat respectively, and in breast they ranged 0.586- 14.380 and 1.174-11.900$\times10^4$CFU/ g meat respectively. The psychrophiles count in whole chicken carcasses ranged 1.950- 13.740$\times10^4$ and 0.900-27.420$\times10^4$ CFU/ g meat, in thighs they ranged 0.554-8.560 and 0.360- 8.000$\times10^4$ CFU/ g meat, and in breast ranged 0.734-8.880 and 1.140- 9.360 $\times10^4$ CFU/ g meat in batches 1 and 2 respectively. Psychrotrophes count in whole carcasses ranged 1.964- 14.280 and 1.286- 21.800$\times10^4$ CFU/ g meat, in thighs they ranged 1.560- 12.640 and 3.42- 7.680 $\times10^4$ CFU/ g meat, and in breast ranged 0.770- 13.140 and 1.178- 8.440 $\times10^4$ CFU/ g meat in batches 1 and 2 respectively. The most probable number (MPN) of both total coliforms and fecal coliforms in the two batches were less than 1 CFU/ g meat in all samples that inspected. Significant differences were recorded among all marks and between both batches for each mark including the three types of meat while all samples were *E. coli* O157: H7, coagulase positive staphylococci, and *Salmonella* free.

KEY WORDS: Bacterial quality, Standard plate count, Psychrophil count, *E. coli* O157:H7, *Staphylococcus, Salmonella.*

*** This research is extracted from a PhD dissertation of the first researcher.**

Introduction

Chicken and poultry products have become popular due to their specific sensory attributes and the increasing tendency of the public to consider white meat as being healthier compared to red meat (Geonaras *et al*., 1995). The presence of pathogenic microorganisms, spoilage microorganisms, or both in poultry is undesirable but unavoidable and lead chicken meat to be deteriorated in quality (Brown, 1982; Russell, 2001). Meat cannot be without microorganisms but their number can be increased by providing the optimum temperature, humidity, and oxygen leading to spoilage while the microbial growth will be static and limited at frozen temperatures (Inoue & Ishikawa, 1997; Nollet, 2007; Biswas *et al*., 2011). In Iraq, Zangana (2006) noticed that the total count of Psychrophilic bacteria in frozen chicken thigh were 4.70 – 4.28 log./ gm meat and the mean value of coliform count in frozen chicken thighs were ranged between 3.86- 4.86 log/ g meat. Alrubaei *et al*. (2007) found that the psychrophilic count of broiler chicken meat stored at 4C° for 0, 3, 6 days were 6.1, 6, 6.5 log / gm meat respectively while Al-Mosawy *et al.* (2008) found that the psychrophilic count in minced breast chicken meat stored at 4C°for 0, 3, 6 day were 6.0, 6.2, 6.5 log/ gm meat respectively. Poultry are highly susceptible to infection with *E. coli* 0_{157}:H_7 (Beery *et al*., 1985; Stavric, *et al*., 1993). Contamination of poultry carcasses with enterotoxigenic *Staphylococcus* isolates of human origin often occurs at processing (Adams & Mead, 1983). In the neighbor Saudi Arabia (KSI), Zeitoun, & Al-Eid (2003) mentioned that two of five marks of imported frozen chicken collected from Al-Hassa revealed no coagulase positive staphylococcal count whereas *Salmonella* was positive in some, while Al - Dughaym & Al-Tabari (2009) mentioned in their study on 10 samples of chicken thighs collected from Al-Ahssa market, that the total mean value of *Staphylococcus aureus* was less than 10^2 CFU/g meat. Foods from animal's origins (especially poultry) are important source of human *Salmonella* infections, especially those which may become contaminated during handling by an ill patient or carrier persons (Gast, 2003). In poultry the cross contamination

is also most likely to occur during evisceration procedures with the use of plucking machines, in which these mechanical devices often rupture the abdominal air sacs and pericardium of the affected birds (Herenda & Franco, 1996 and Lake *et al*., 2002). AL-Dughaym and Altabari (2009) in KSA ranked the samples from carcass cuts (chilled, frozen, fillet and thigh) to minced meat or further processed products as burger, nuggets, frankfurter and meat paste loaf and revealed *Salmonella arizona* was isolated at once from thigh samples. In Kurdistan region of Iraq, the demand for meat increased during the last decades (Iraqi poultry and product annual, 2010), this led poultry meat of different sources to be imported in high quantities. Without regarding the formal inspection may or may not be occur. So, the aim of this study was to assess the bacteriological load of imported frozen chicken meat trademarks available in Sulaimani markets in order to determine their quality for human consumption.

Materials and methods

Sampling: The ultimate inspection included a total number of (360) samples of frozen chicken meat belonged to 7 foreign trademarks were collected in two batches from different parts of Sulaimani city markets. The samples consisted of whole chickens (160 samples- 80 from each batch), thighs (120 samples- 60 from each batch) and breasts (80 samples- 40 each batch). The duration of sampling batch (1) was from the first of February till end of April 2010, while batch (2) was sampled from July till end of September 2010. The whole chicken carcasses were distributed on 40 samples for each of DMIS (Iranian), Gedik (Turkey), Sadia (Brazilian) and Frinal (Brazilian) while thighs were on 40 samples of Sadia (Brazilian), 40 samples of Tyson (American) and 40 samples of AJC (American), and the breasts were from Sadia (Brazilian), 40 samples of Seara (Brazilian). From each trademark (40) samples (whole carcasses, thighs or breasts) were collected and the whole carcass or a number of meat specimens from each sample were obtained aseptically. Both of AJC and Tyson marks were sold in unpacked condition at retail selling. All Samples were transferred inside a Cork chilled box (ice box) to the laboratory of Animal production department, Agriculture faculty, Sulaimani University, as soon as possible to prevent thawing.

-pH measurement

The procedure of **Naveena and Mendiratta (2001)** was used to determine the pH of the specimens

Microbiological tests

The procedure of USDA/ FSIS (1998) was used, aseptically, 50 ± 0.1 g of the sample (Major and minor pectoralis with femur muscles) were used.

Total plate count

The procedure of AOAC 966.23 C (1995) was used.

Psychrophilic bacterial count (APHA, 1992)

The same procedure for counting was used as in Total Plate count; 1ml of the series dilutions, then the plates were incubated at 5-7ºC for 10 days.

Psychrotrophic bacterial count (AOAC, 1995)

The same protocol of pour plate procedure was used, plates were incubated at 20ºC for 4-5 days.

Most Probable Number (MPN) of Coliforms (USDA/FSIS, 1998; Garthright, 1995)

The procedures of 3 set tubes were used. To count the MPN of the fecal source coliforms, a loopful from the positive tubes was inoculated into tubes with lactose broth and incubated at 45.5 ± 0.05°C in covered water bath for 24 ± 2h. The positive Coliform tubes were having acid (change the colour of medium to yellow) and gas. The number of the positive tubes that incubated at 35ºC was considered in the calculation of MPN of total coliforms, while that positive at 45.5ºC was considered to calculate the fecal source (thermotolerant) coliforms. MPN/g of meat was calculated by comparing the positive tubes against standardized MPN tables (USDA/FSIS Laboratory guide book, 2008). The highest lactose positive dilution tubes were confirmed to contain fecal source Coliforms (*Escherichia coli*) (APHA, 1992) by streaking onto Eosin–Methylene Blue agar (EMB) plates, after incubation at 37°C for 24 hours, the plates were examined for typical *E. coli* colonies, which are metallic green sheen. A number (2-3) of isolated colonies were picked out and purified on EMB, then subculture to Nutrient agar slants and incubated at 35ºC for 18 -24 hours to be tested completely. The completed test was achieved by sending the nutrient agar slants to be identified at the species level as *E. coli* through an automated Vitek- 2 system at the Central Health Laboratory, Erbil city, after a

gram stain was done for the slants; those gram negative rods were send.

Identification of *E. coli* O157:H7: Those *E. coli* detected by MPN were completely identified by Vitek 2system which identifies the enteropathogenic strain *E. coli* O157; H7 by development of agglutination with a normal saline suspended colonies.

Identification of Coagulase positive Staphylococci (*Staphylococcus aureus*) (Bennet & Lancette, 1998)

Performed by using Baired-Parker medium (Prondisa, Spain), and also by using Mannitol salt agar, which was prepared according to manufacturer directions (APHA, 1992). Coagulase test was achieved according to Collins & Lyne (1987). The black colonies were surrounded by a clear zone on Baired-parker agar, yellow mannitol fermentative on mannitol salt agar, and clotted the rabbit blood plasma within four hours were presumptively identified as coagulase positive *Staphylococcus aureus,* but for the purpose of complete identification, a number of colonies that showed positive and negative results were purified by streaking on mannitol salt agar and incubated for 24h at 37◦C, and then isolated colonies were sub cultured onto Nutrient agar slants, incubated at the same conditions and were sent to be completely identified by Vitek2 system in Erbil city to be identified at the species level.

Identification of *Salmonella*

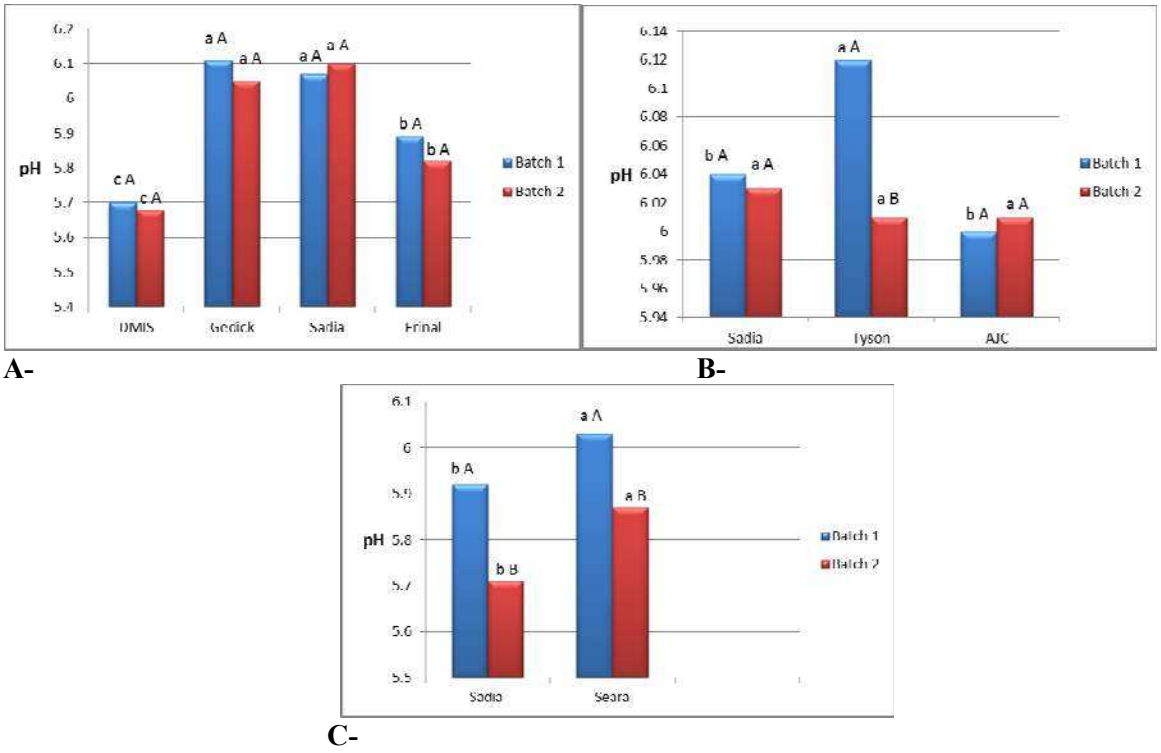
The procedure of Waltman (1999) and Talaska (2004) were used. Three to five suspected colonies were inoculated separately into TSI (Triple Sugar Iron) agar (Merck, Germany) and Urea agar (Biolofe, Italy) and incubated at 35°C for 24 hours, the colonies with characteristics reaction of *Salmonella* were subcultured in duplicates on Nutrient agar slants, incubated at 37°C for 24 hrs and stained by gram staining. The duplicates of the gram negative ones were transferred to the Central Health Laboratory (Erbil) to complete identification by Vitek2 system and to the *Salmonella* National Center in Baghdad city as well. The isolates that gave negative Urea test, and TSI test gave Acid, gas (Yellow, pink, black with gas formation), the biochemical tests of automated Vitek2 system, and the results obtained from Salmonella National Center, all were confirmative for the presence of *Salmonella* spp. in the samples.

Results

pH values: Figure (1) showed the pH mean values of two batches for all inspected marks. In whole carcasses the values ranged 5.70- 6.11 for batch (1) and 5.68- 6.10 for batch (2). Gedik and Sadia did not significantly differ in both batches, while these marks were significantly difference ($p< 0.05$) for both Frinal and DMIS which in turn were different ($p< 0.05$) in between. Also, there were no significant differences between the two batches for each mark. The pH mean values of two batches of chicken thighs ranged 6.00-6.12 for batch (1) and 6.01-6.03 for batch (2). Tyson was significantly different ($p< 0.05$) from the other two marks. All thigh marks showed no significant difference between two batches except for Tyson. The same figure showed the pH mean values of two breast trademarks which ranged 5.92-6.03 and 5.71-5.87 for batch(1 and 2) respectively. Both marks revealed significant differences ($p< 0.05$) in both batches. Also, both batches were significant difference ($p< 0.05$) for each mark.

Bacterial count: The mean values of total bacterial counts of the whole carcasses of two batches for the four trademarks are shown in table (1), the mean values of standard plate count showed significant differences ($p< 0.05$) among all marks for both batches, also, there was a significant difference ($p<0.05$) between the two batches for each mark. All trademarks are within the standard levels of Iraqi quality regulations for frozen poultry meat (ICOSQC- IQS 2270/4, 2006) that specified the SPC of frozen poultry between 10^5- 10^7 CFU/g meat. Mean values of total plate count for the thighs in two batches of four trademarks are shown in table (2). The mean values for all marks were significantly different in both batches ($p< 0.05$). There were significant differences between both batches for each mark ($p< 0.05$), so, all trademarks matched standard levels in Iraqi quality regulations (ICOSQC IQS 2270/4, 2006). The mean values of total bacterial counts of the breasts for two batches of two trademarks are shown in table (3). The mean values for both marks were significantly different ($p< 0.05$) in each batch. There was no significant difference between the two batches for Seara, while for Sadia there was ($p< 0.05$). All trademarks matched the standard levels of Iraqi quality regulations for frozen poultry meat (ICOSQC /IQS 2270/4, 2006).

The psychrophilic bacterial count mean values of the carcasses of two batches for four trademarks are shown in table (1). The mean values showed that there was no significant difference between the two batches of both Gedik and Frinal while there was significant difference ($p< 0.05$) between the two batches for both of DMIS and Sadia. The mean values of psychrophilic bacterial count of the thighs are shown in table (2).The results revealed that all marks, in both batches (1, 2), appeared significantly different ($p< 0.05$). There were significant differences between the two batches for each mark. The mean values of psychrophilic bacterial count of frozen breast are shown in table (3). The results showed the presence of significant difference ($p< 0.05$) between the two marks in both batches. The mean values of psychrotrophic bacterial count of whole carcasses are shown in table (1). There were significant differences ($p< 0.05$) between the two batches for both of DMIS and Sadia while both of Frinal and Gedik were not different significantly. The mean values of psychrotrophic bacteria count of the thighs are shown in table (2). All marks significantly differed ($p< 0.05$) in both batches. Also, there was a significant difference ($p< 0.05$) between two batches for each mark except for AJC mark which showed no difference. The mean values of psychrotrophic in the breasts are shown in table (3). There was a significant difference ($p< 0.05$) between the two marks for both batches. Also, there was significant difference ($p< 0.05$) between the two batches for each mark.



A- **B-**

C-

Figure (1): pH mean values of all chicken trademarks: A-Whole chicken carcasses. B- Chicken thighs. C- Chicken breasts.

-Means having different lower-case at the same row (Trademarks) and upper – case at the same column (Batch) are significantly different.at ($p < 0.05$).

The mean values of most probable number (MPN) of total coliform count of whole carcasses are shown in table (1). There were no significant differences among all marks except for Gedik in both batches, also, there was no significant difference between the two batches for each mark. While the MPN of total fecal coliforms of whole carcasses for two batches of four trademarks are shown in table (2). Batch (1) showed that all marks were not significantly different except for Gedik, batch (2) showed that both DMIS and Frinal were significant difference ($p< 0.05$) with Gedik and Sadia which were not varied in between. *E. coli* were isolated from both batches of Gedik and Sadia carcasses (2). The microbiological analysis results for whole chicken carcasses matched standard levels of Iraqi quality regulations for frozen poultry meat (ICOSQC IQS 2270/4, 2006).

Table (2) showed the MPN of total coliforms mean values of chicken thighs and there were no significant differences ($p< 0.05$) among all marks for both batches. Also, there was no significant difference between the two batches for each mark. The MPN of total fecal coliforms of chicken thighs are shown in table (2). There were no significant differences among all marks in both batches and no significant difference between two batches for each mark.

The mean values of MPN total Coliform count of the breasts are shown in table (3), there was a significant difference ($p< 0.05$) between the two marks in both batches. There was no significant difference between the two batches for each mark. There were no significant differences between two marks for each batch. *E. coli* and their enteropathogenic variant O_{157}:H_7 were not detected in breasts of all trademarks inspected. There were no significant differences with respect to MPN of coliforms among the four inspected marks for whole carcasses except for Gedik (table, 1), which was higher than other marks.

Table (1): Bacterial counts mean values of imported whole frozen chicken carcasses for two batches of four trademarks in (CFU/ g meat).

Traits	Bat-ch	Trade mark			
		DMIS	Gedik	Sadia	Frinal
Total plate count	1	$0.54 \times 10^4 \pm 0.24$ d A	$5.72 \times 10^4 \pm 0.22$ a A	$3.18 \times 10^4 \pm 0.07$ c B	$3.90 \times 10^4 \pm 0.26$ b B
	2	$0.26 \times 10^4 \pm 0.16$ d B	$3.42 \times 10^4 \pm 0.15$ c B	$4.72 \times 10^4 \pm 0.53$ b A	$5.72 \times 10^4 \pm 0.23$ a A
PSP. bacterial count	1	$1.95 \times 10^4 \pm 0.34$ c A	$13.74 \times 10^4 \pm 1.58$ a A	$7.60 \times 10^4 \pm 0.64$ b B	$12.16 \times 10^4 \pm 1.68$ a A
	2	$0.90 \times 10^4 \pm 1.33$ c B	$10.11 \times 10^4 \pm 0.55$ b A	$27.42 \times 10^4 \pm 3.97$ a A	$15.02 \times 10^4 \pm 0.83$ b A
PST. bacterial count	1	$1.96 \times 10^4 \pm 0.73$ b A	$14.28 \times 10^4 \pm 1.11$ a A	$10.54 \times 10^4 \pm 0.94$ a B	$9.50 \times 10^4 \pm 2.67$ a A
	2	$1.28 \times 10^4 \pm 1.16$ c B	$11.62 \times 10^4 \pm 0.66$ b A	$21.80 \times 10^4 \pm 2.67$ a A	$14.22 \times 10^4 \pm 1.56$ b A
MPN of total Coliforms	1	< 0.03 ± 0.0 b A	0.258 ± 0.08 a A	0.030 ± 0.0 b A	< 0.03 ± 0.0 b A
	2	<0.03 ± 0.0 b A	0.132 ± 0.04 a A	0.030 ± 0.0 b A	< 0.03 ± 0.00 b A
MPN of total fecal coliform	1	< 0.03 ± 0.0 b A	0.101 ± 0.09 a A	0.030 ± 0.0 b B	< 0.03 ± 0.0 b A
	2	< 0.03 ± 0.0 c A	0.101 ± 0.09 a A	0.074 ± 0.0 b A	< 0.03 ± 0.0 c A
E.coli	1	ND	ND	ND	ND

(O_{157} :H_7)	2	ND	ND	ND	ND
Coa. pos. *St. aureus*	1	ND	ND	ND	ND
	2	ND	ND	ND	ND
Salmonella Spp.	1	ND	ND	ND	ND
	2	ND	ND	ND	ND

- Means having different lower-case at the same row (Trademarks) and upper – case at the same column (Batch) are significantly different at ($p < 0.05$).
- ND : Not detected. - The symbol (<) was not included in statistical analysis.

Table (2): Bacterial counts mean values of imported frozen chicken thighs for two batches of three trademarks (CFU / g meat)

Traits CFU/g meat	Batch	Trade mark		
		Sadia	Tyson	AJC
Total plate count	1	3.0×10^4 ± 0.53 b B	17.52×10^4 ± 0.29 a A	1.17×10^4 ± 0.36 c A
	2	5.82×10^4 ± 0.22 b A	12.50×10^4 ± 0.70 a B	0.73×10^4 ± 0.35 c B
Psychrophilic bacterial count	1	4.62×10^4 ± 0.51 b B	8.56×10^4 ± 0.31 a A	0.55×10^4 ± 0.46 c A
	2	8.00×10^4 ± 0.39 a A	6.28×10^4 ± 0.30 b B	0.36×10^4 ± 0.58 c B
Psychrotrophic bacterial count	1	1.56×10^4 ± 0.31 b B	12.64×10^4 ± 0.29 a A	6.46×10^4 ± 0.78 c A
	2	3.42×10^4 ± 0.18 b A	7.68×10^4 ± 1.22 a B	4.82×10^4 ± 0.21 c A
MPN of total coliforms	1	0.03 ± 0.0 a A	0.03 ± 0.0 a A	< 0.03 ± 0.0 a A
	2	0.03 ± 0.0 a A	0.03 ± 0.0 a A	< 0.03 ± 0.0 a A
MPN of total fecal coliforms	1	0.03 ± 0.0 a A	< 0.03 ± 0.0 a A	< 0.03 ± 0.0 a A
	2	0.09 ± 0.0 a A	0.03 ± 0.0 a A	< 0.03 ± 0.0 a A
E.coli (O_{157} H_7)	1	ND	ND	ND
	2	ND	ND	ND
Coa. Pos. *St. aureus*	1	ND	ND	ND
	2	ND	ND	ND
Salmonella Spp.	1	ND	ND	ND
	2	ND	ND	ND

Means having different lower-case at the same row (Trademarks) and upper- case at the same column (Batch) are significantly different at ($p < 0.05$).
ND: Not detected. The symbol (<) was not included in statistical analysis.

Table (3): Bacterial counts mean values of imported frozen chicken breast for two batches of two trademarks(CFU / g meat).

Traits	Batch	Trade mark	
		Seara	Sadia
Total plate count	1	$14.38 \times 10^4 \pm 1.20$ a A	$0.586 \times 10^4 \pm 0.56$ b B
	2	$11.90 \times 10^4 \pm 0.99$ a A	$1.174 \times 10^4 \pm 0.53$ b A
Psychrophilic bacterial count	1	$8.88 \times 10^4 \pm 0.67$ a A	$0.734 \times 10^4 \pm 0.74$ b B
	2	$9.36 \times 10^4 \pm 0.59$ a A	$1.140 \times 10^4 \pm 0.62$ b A
Psychrotrophic bacterial count	1	$13.14 \times 10^4 \pm 0.36$ a A	$0.77 \times 10^4 \pm 1.36$ b B
	2	$8.44 \times 10^4 \pm 1.04$ a B	$1.178 \times 10^4 \pm 0.13$ b A
MPN total Coliforms count	1	0.030 ± 0.0 a A	4.58 ± 0.26 a A
	2	0.030 ± 0.0 a A	2.44 ± 0.21 a A
MPN total fecal coliforms	1	< 0.030 ± 0.0 a A	0.03 ± 0.0 a A
	2	< 0.030± 0.0 a A	0.03 ± 0.0 a A
E.coli (O_{157} :H_7)	1	ND	ND
	2	ND	ND
Coa. Pos. *St. aureus*	1	ND	ND
	2	ND	ND
Salmonella Spp.	1	ND	ND
	2	ND	ND

Means having different lower-case at the same row (Trademarks) and upper-case at the same column (Batch) are significantly different at ($p < 0.05$).
ND: Not detected. The symbol (<) was not included in statistical analysis

Discussion

The differences in pH mean values among the whole carcasses (except for both Gedik and Sadia marks), could be due to the stress factors before slaughtering which resulted in drop of glycogen content in meat and less lactic acid formation (Berri, *et al*., 2005), or exhaustion of the bird before slaughtering (Owens & Sam, 2000). The decrease in pH value could be due to liberation of free fatty acids as a result of the lipolytic enzymes, either because of prolonged storage period, or bad storage conditions (Frazier & Westhoff, 1988 and Sams, 2001) which may explain why DMIS mark gave a low pH value. The study results were similar to results of Ali & Al-Zahran (2010) who recorded the pH values in chicken carcasses stored at chilling conditions for 0, 2, 4 and 6 days as 6.00, 6.21, 6.80 and 7.20 respectively. The same explanations above are true to elucidate the differences in pH values in inspected thighs and breasts. The pH values obtained in this study were in agreement with those obtained by Mead (2000) who stated that leg and breast muscles of chicken have pH

values range of (6.1–6.4) and (5.6-5.8) respectively, whilst they were lower than those recorded Al- Hamadany (2009) who recorded the range of pH values in imported frozen chicken thighs sold in Baghdad's markets as 6.60 - 6.90.Unfortunately, there is no related standard for pH in Iraqi quality regulations to be compared with.

Although there were significant differences in total plate count among the inspected carcasses from the collected marks, Gedik had the highest count while DMIS had the lowest. This could be due to many reasons,

the low pH value for DMIS (figure, 1) may had an influence on bacterial growth (Jay *et al.*, 2005), other factors occur through poultry manufacturing processing like slaughtering, eviscerating, scalding, chilling, packaging, freezing, transporting and handling (Bolder, 1998). Also, as being mentioned by ICMSF (2011) that the rate of spoilage is influenced by many factors such as, storage temperature, initial number and type of microorganisms when packaged, type of packaging and chemical composition. The same table revealed significant differences in count between two batches of both Sadia and Frinal marks. The count in batch (2) appeared little higher than batch (1), this may be due to the effect of season; in summer the electric current cut off affects the thawing and refreezing of the products which in turn lead to increase in count as mentioned by Narasimha *et al*. (1998), while DMIS and Gedik appeared not to be affected seasonally because they have been stored in different freezing conditions as they were less demanded by consumers than the other two marks. Indeed, the freezing process inhibits mesophilic bacterial growth (Jay *et al*., 2005). Bailey *et al*. (2000) recorded higher bacterial count than the current study as they reported the mean total plate count in chicken carcasses stored in 4 ºC for 7 days, for 0 till 7 days as 4.6 to 6.6 log CFU/ g meat respectively while their results remained the same when the samples stored by chilling to -18 ºC. Morshedy & Sallam (2009) recorded the total plate count in chicken carcasses stored at 4ºC for 0, 4, 6, days as 5.13, 6.87, 7.4 $\log^{10}$ CFU/g meat respectively. It should be kept in mind that the above two articles used fresh meat but not frozen. Freezing has lethal effect on some microorganisms by formation of ice crystals (Kuntz, 1996) and as a result the count decreased.

The highest psychrophilic count in batch (1) of Frinal and batch (1) of Gedik mark, batch (2) of the Brazilian Sadia, could be due to many reasons, the different manufacturing steps (slaughtering, scalding, defeathering, chilling, handling, packaging) all these factors lead to adding more bacterial count (Bolder, 1998), and the initial bacterial count had an effect on the final count (ICSMF, 2011). Mead (2004) and Jay *et al*. (2005) had mentioned increasing the psychrophilic count within long storage at low temperature. The significant difference ($p< 0.05$) in psychrophilic count between two batches of DMIS and Sadia marks may be due to the seasonal effect on the products, in appropriate storage temperature or fluctuation of storage temperature are the most causes of spoilage (Russell, 2009). The same table showed that the mean value of psychrophilic bacterial count of all inspected marks were higher than total plate count (mesophilic bacteria), this may approve that the increase of count might be due to the contamination of freezers or the storage area with that kind of microorganisms specially *Pseudomonas* spp. or due to the thawing and refreezing processes through processes of marketing handling etc., which lead to rapid spoilage of the products during thawing (Russell, 2001 and Jay *et al*., 2005).

In table (2) which explained the mean value of psychrophilic count in thighs the results showed that AJC mark had the lower psychrophiles count in both batches, this could be due to good manufacturing practices which has a role in decreasing the count (Bolder, 1998 and Mead, 2004) which may be an advantage for those marks with low psychrophilic count. The results obtained here were little lower than those recorded by Al-Hasnawi (2010) who evaluated the quality of thighs meat of imported frozen chicken in Diwaniya city; she claimed that the psychrophilic bacterial count for Tyson thighs as 55.6×10^4 CFU/g meat while for Sadia thighs as 10.6×10^5 CFU/g meat. The current results were also lower than results published by Al-Hamadany (2009) who recorded psychrophilic bacterial count mean value in Sadia chicken thighs collected from Baghdad markets were 10.6×10^5 CFU/g meat, when the average count of other six imported and local marks ranged between 3×10^2 and 2×10^6 CFU/g meat.

The results related with psychrophilic bacterial count mean values of the breasts samples (table, 3) which revealed a rather high count in Seara mark in comparison to Sadia mark for both batches, may explain the role of

several factors have been mentioned before, including the number of microorganisms that contaminate the carcasses through slaughtering, handling, and marketing processes (Bolder, 1998 and Mead, 2004), which differ between the two marks according to the manufacturing processes. The results here agree with those reported by Kenawi *et al*. (2007) who found that the psychrophilic bacterial count in breasts stored at 4ºC in day 1 to 24 increased from 3 log CFU/g meat to 6.8 log CFU/g meat respectively, and results of Gallas *et al*. (2010) who reported that the psychrophilic count in chicken breasts stored under refrigerator condition at 0, 3, 9, 14 days were 3.1, 2.7, 4.4, 6.5 $\log^{10}$ CFU/g meat respectively. Despite the claim of Abu-Ruwaida *et al*. (1996) who studied the microbial shelf life and quality of frozen broiler chickens and reported that prolong frozen storage did not cause substantial changes in the bacterial count of carcasses stored at (-12ºC), as being decreased slightly when stored at (-18ºC), but Modi *et al*. (2005) and Sudheer *et al*. (2011) disapprove that when they reported increasing in psychrophilic count in prolong freezing storage. Even (ICMSF, 2011) confirmed that frozen poultry typically does not undergo microbial spoilage, but they also determined that storage temperature should be controlled to prevent fluctuation which directly have an effect on microbial growth.

The mean values of psychrotrophic bacterial count revealed the lowest by DMIS (table, 2), this could be due to the lowest total bacterial count and psychrophilic. Season may be a strong factor in showing significant differences between the two batches of all marks, due to electrical cut out, freezing and thawing process lead to reactivation of psychrotrophic microorganisms especially in samples collected from grosser shops. Psychrotrophic counts were higher than the mesophilic (table, 1), this mean that most of the mesophiles were adapted to grow in low temperatures (Kraft, 1992; Zeitoun & Al-Eid, 2003; and Jay *et al.*, 2005) as well as a number of psychrophiles that grew in the incubation temperatures of psychrotrophic ones as has been confirmed by several researches (Olson & Nottingham, 1980; Moyer & Morta, 2001 and Jay *et al*., 2005), indeed the storage area and the time of storage may serve as an additional factor (Mead, 2004 and Russell, 2009). The lowest mean value of psychrotrophic bacterial count in the thighs of both batches for the three marks that have been inspected was scored by AJC mark, while Tyson showed the highest in both batches (table, 2). The Psychrotrophes were lower than total aerobic bacterial count, because the samples were bought fresh and the products did not stay for long periods in shops and markets therefor the psychrotrophic bacteria did not have enough time to increase (Jay *et al*., 2005). Tyson mark in batch (1) had a higher count than batch (2) which may be due to the exploration mode of the products in winter (as mentioned earlier) which may led to the growth of mesophilic bacteria at thawing; during refreezing the mesophiles adapt to be psychrotrophs to handle low temperatures (Mead., 2004; Jay *et al*., 2005). The mean value of Psychrotrophic bacterial count of the breasts were higher in batch (1) of Seara (table, 3) which may again be due to the high mesophilic count (total plate count) that has been detected in Seara regarding that most of mesophiles turned to Psychrotrophic and Psychrophils at low temperatures (Lahellec *et al*., 1975; Jay *et al*., 2005). In contrast, samples of the batch (2) of Sadia mark recorded higher count than batch (1) which reflects the effect of summer heat leading to increase in the psychrotrophic count in addition to the high mesophilic count shown in batch (2) of the Sadia. To debate this, some facts should be kept in mind, that Psychrotrophic and Psychrophils have the same minimum temperature ranged -5 to 5 °C but, psycrotrophes have optimum growth temperature at 25 to 30 ºC and Psychrophils have 12-15 ºC, as an optimum range (Olson & Nottingham, 1980; Moyer & Morta, 2001 and Jay *et al*., 2005), and psychrotrophes has been defined as subgroup of mesophiles since their optimum temperatures for growth are in moderately low range and they still grow well at temperatures below the maximum for most mesophiles (Kraft, 1992; Bolder, 1998). Some strains of species that fall into the indicator organisms categories (such as certain coliforms and *Enterobacter* from the family *Enterobacteriaceae*) are psychrotrophic and can multiply on refrigerated raw poultry carcasses and products (ICMSF, 1986).

Some of pathogenic and food poisoning microorganisms are also psychrotrophic in nature like *Salmonella* species*, Staphylococcus, E.coli, Listeria* (Sams, 2001; Mead, 2004 and Jay *et al*., 2005). So, in food quality it's important to count the psychrotrophic bacteria, because any increase may lead to more pathogenic bacterial isolation and identification (USDA /FSIS, 1998).

The sources of coliforms were reported to be due to process of slaughtering, handling and marketing (Mead *et al*., 1993 and Sarlin *et al*., 1998), and the good manufacturing practices (GMP) in addition to that, HACCP may be concerned in manufacturing countries, to control the processing practice (Mead *et al*., 1993; Donald *et al*., 2001), and reducing of count may be due to controlling processes of slaughtering, eviscerating, scalding, chilling, packaging, freezing, transporting and handling (Bolder, 1998). Yet the MPNs for all marks were very low in comparison to results recorded by Khalifa & Abd El-Shaheed (2005) who reported the coliform count in 35 raw chicken samples as $4.1.x10^3$ CFU/g meat and results of Zeitoun and Al-Eid (2003) who studied the safety and quality of frozen poultry at AL-Hassa supermarkets in Saudi Arabiya; 360 samples from seven locally produced companies and from five imported companies were tested, the mean value of coliform count in imported chicken was 1.00 CFU/cm^2.

Table (2) showed count in the frozen thighs, there were no significant differences in the mean value of MPN of coliforms among all the three marks in both batches. This confirms the good hygienic practices by the manufacturers' inspite of that both Tyson and AJC marks were unwrapped at retail outlet. Hygienic habits of the vendors including freezing process may be an additional factor on coliforms count reduction (Bailey *et al*., 2000). Al- Hamadany (2009) reported similar results; she recorded that the frozen chicken thighs of Sadia mark in both retail and whole outlet were coliform free, but the results of AL- Hisnawi (2010) who found that the total coliform count in 15 samples of Sadia mark thighs was 7.3×10^3 CFU/ g meat and in 15 samples of Tyson thigh mark was 6.6×10^3 CFU/ g meat, AL- Hisnawi attributed this difference to bad manufacturing and handling of the products. Although, there were no significant differences in MPN of coliforms between the breasts of the two marks inspected (table, 3). So, the presence of coliform is an indicator of unsanitary conditions through slaughtering processes specially evisceration (Donald et *al*., 2001), or contaminating of the products during handling specially at manufacturing (Mead, 2004) because the products were packaged at retile outlets. There were no significant differences also between the two batches for each mark, which shared the same hygienic and handling conditions. Since there was no specific coliform criteria in the Iraqi quality regulations specified for frozen chicken meat, so the comparison with limits given by H.P.F.B. (2003) which determined coliform limit at $10\text{-}10^3$ CFU/g meat and ICMSF (1986) determined it at 10^2 CFU/g for meat, revealed that all marks for whole chicken and chicken portions were within the standard limits.

As shown in tables (1, 2, 3), *E.coli* O157: H7 was not detected in all inspected samples. This serovar is considered as one of the causatives of food born disease (Feng *et al*., 2001 and Mead, 2004) and so should be included in inspection. The current result is not an exception in this respect if we know that *E.coli* O157 H7 was not applicable in results published by USDA-FSIS (1996) out of 1297 postchill carcass rinse samples from approximately 200 broiler processing plants. *E.coli* is not included in the specific Iraqi standards in both IQS 2270/4 and 3725/4 (Microbiological limits of frozen chicken) (ICOSQC, 2000 and ICOSQC, 2006), whilst all the inspected marks were within the limits introduced by ICMSF (Microbial limits of fresh poultry) (1986) which specified that any food products should be free of this kind of bacteria.

Coagulase positive *Staphylococcus aureus* were not detected in all samples for two batches of all marks (table,1, 2, 3). There are no standard for the incidence of Coagulase positive *Staphylococcus aureus* in Iraqi quality regulations to be compared with (ICOSQC, 2000; ICOSQC, 2006). This was agree with Al-Hamadany (2009) who recorded that the count of *Staphylococcus aureus* in most of imported frozen thighs sold in Baghdad were zero. Contamination of poultry carcasses with enterotoxigenic *S. aureus* of human origin often was mentioned to occur during processing (Adams & Mead, 1983). Indeed coagulase-positive *S. aureus* is typically originated from humans (Donald *et al*., 2001). Even though, both Tyson and AJC marks were unpackaged at retail selling. The effect of freezing on the microorganisms may be the major reason for lowering coagulase positive *staphylococcal* count (Lambert *et al*., 1991 and Jay *et al*., 2005). In a similar study, Al-Hamadany (2009) mentioned that the mean count of *Staphylococcus aureus* in Sadia mark thighs was zero CFU/ g meat in whole sells, and 1×10^2 CFU/g meat in retail sells. While Al -Dughaym & Al-Tabari (2009) in Saudi Arabiya, mentioned in their study on 10 samples of chicken thighs

collected from Al-Ahsa market, that the total mean value of *Staphylococcus aureus* was less than 10^2 CFU/g meats.

Salmonella were also undetected in all samples inspected (Tables, 1, 2, 3), the no detection of *Salmonella* agreed with standard levels of Iraqi quality regulations for frozen poultry meat (ICOSQC IQS 2270/4, 2006).

This means good manufacturing conditions, handling and transporting for the products (Gast, 2003; Sampathkumar *et al*., 2003 and Maed, 2004). The use of prebiotic and probiotic additives to birds feed had been reported to have a good effect on bacterial growth in the birds intestine (Wolfeden *et al*., 2007), but in any case FSIS in 1996, has reported that many produced countries follow the Pathogen Reduction-Hazard Analysis Critical Control Point (HACCP) systems, which set pathogen reduction performance standards for *Salmonella* for slaughter plants and for plants producing raw ground meat products (Bilgili, 2001). Yet freezing had low effect on the *Salmonella* microorganisms (Mead, 2004; ICSMF, 2011), since the last reference reported that Salmonella can live in a frozen product for about one year. AL- Hamdany (2009), in Iraq, reported compatible result when revealed all inspected sample were *Salmonella* free.

Conclusion

Regarding the microbiological quality, all chicken samples (whole chicken carcasses, thighs and breasts) were within Iraqi and international standards and fortunately all samples were free from *Salmonella, E.coli* O15:H7 and coagulase positive *Staphylococcus aureus.* In spite of that there are no standard limits for psychrophilic bacterial count, Psychrotrophic bacterial count, Coliform count, Staphylococcus bacteria, in Iraqi quality standards to be compared with.

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جۆرایە تی بکتیری لە مریشکی هاوردەی بەستوو لە بازاڕەکانی شاری سلیمانی

پوختە

بۆ هەلسەنگاندنی جۆرایەتی بکتیری بۆ گۆشتی مریشکی بەستووی هەوارده کە لە بازارەکانی شاری سلیمانیدا هەیە،لە کۆی ٣٦٠ نموونە مریشکی بەستوو کە حەوت مارکەی بازرگانی لە خۆ گرتبووبە دوو تاوەوە(باج)، لاشە تەواوەکانی مریشکەکان ١٦٠ نموونە بوون، ٨٠ یان لە تاوی یەکەم وە ٨٠ لە تاوی دووەمدابوون. رانەکان ١٢٠ نموونە بوون (٦٠ بۆ هەر تاوێك)، سنگەکانیش ٨٠ نموونە بوون(٤٠ بۆ هەر تاوێك) کە لە بازارەجیاوەزەکانی شاری سلیمانی کۆکرانەوە. ئەژمارکردنی بکتیری گشتی لە باجی یەکەم ودووەمدابۆ لاشەتەواوەکان بە مەودای ٥,٧٢-٠,٥٤ × ١٠^٤ و ٥,٧٢- ٠,٢٦ × ١٠^٤ یەکەی پێکهێنانی نیشینگە / گم گۆشت. لە رانەکانیشدا بە مەودای ١,١٧ -١٧,٥٢ و٠,٧٣- ١٢,٥٠ ×١٠^٤ یەکەی پێکهێنانی نیشینگە / گم گۆشت تۆمارکرد لە هەردوو باژەکان یەکەم و دووەمدا یەك لەدوای یەك. مەودای ئەژمارکردنی لە سنگدا ١٤,٣٨،١١,٩٠ و ١,١٧،٠,٥٨×١٠^٤ یەکەی پێکهێنانی نیشینگە / گو گۆشت لە باچەکانی یەکەم ودووەمدا یەك دوای یەك.

ژمارەی بەکتییریای هۆگری ساردی لە لاشەکانی مریشکی لەش تەواو بەمودای ١,٩٥-١٣,٧٤ و ٠,٩٠- ٢٧,٤٢×١٠^٤یەکەی پێکهینانی نشینگە /گم گۆشت بوو، لە ڕانەکانیشدا ٠,٥٥١-٨,٥٦ و ٠,٣٦-٨,٠٠×١٠^٤ یەکەی پێکهێنانی نیشینگە / گم گۆشت ، لەسنگدا ٠,٧٣-٨,٨٨ و ١,١٤- ٩,٣٦ ×١٠^٤ یەکەی پێکهێنانی نیشینگە / گم گۆشت بوو. ژمارەی بەکتیری بەرگری ساردی لە لاشەکانی مریشکی لە ش تە واو بۆ هەردوو باچە کانی یەکەم و دووەم بەمەودای ١,٩٦-١٤,٢٨ و ١,٢٨-٢١,٨٠ ×١٠^٤ یەکەی پێکهێنانی نیشینگە / گم گۆشت بوو، لە ڕانەکانیشدا٠,٦٤-١٢,٦٤ و ٠,٤٨- ٧,٦٨ ×١٠^٤ یەکەی پێکهێنانی نیشینگە / گم گۆشت لە باچە کا نی یەکەم و دووەمدا ، لە سنگیشدا٠,٧٧- ١٣,١٤ و ١,١٧- ٨,٤٤ ×١٠^٤ یەکەی پێکهێنانی نیشینگە / گم گۆشت لە باچە کانی یەکەمدا و دووەمدا یەك لەدوای یەك بوو. زۆرترین ئەگەر ری ژمارەی بکنیریای قوڵۆن لەهەموو نمونەکاندا بە هەردوو باچە کانەوە لە یەك یەکەی پێکهێنانی نشینگە/گم گۆشت کەمتربوو . هەموو نمونەکان لە هەموو مارکەکاندا بە هەردوو باچە کانەوە بکتیریای *E.coli* O157:H7 , وە جۆرە کانی بەکتیریای Coagulase positive *Staphylococcus aureus* وە بکتیریای *Salmonella* یان تیدا نەبووە.

النوعية البكتريولوجية للدجاج المجمد المستورد المباع في اسواق مدينة السليمانية

الخلاصة

لغرض دراسة نوعية لحم الدجاج المجمد من الناحية البكتريولوجية والمباع في اسواق مدينة السليمانية تم فحص ٣٦٠ عينة من الدجاج المجمد حيث تم اخذها من سبعة علامات تجارية اجنبية جمعت بوجبتين (١٦٠ عينة من الذبائح بواقع ٨٠ عينة لكل وجبة) و١٢٠ عينة من الافخاذ (٦٠ لكل وجبة) و٨٠ عينة من الصدور (٤٠) عينة لكل وجبة. وتم اتباع طرق التحليل العالمية في الفحوصات. اظهرت الفحوصات بان كل عينات الذبائح الكاملة كانت ضمن الحدود الميكروبية القياسية، ووجدت فروقات معنوية بين العلامات والوجبات. سجلت معدلات العد الكلي البكتيري مدى ٠,٥٤-٥,٧٢ × ١٠^٤ و ٠,٢٥- ٥,٧٢ × ١٠^٤ وحدة تكوين مستعمرة/غم لحم في الوجبتين الاولى والثانية على التوالي. والعد الكلي البكتيري للافخاذ كان بمدى١,١٧ -١٧,٥٢ و٠,٧٣- ١٢,٥٠ ×١٠^٤وحدة تكوين مستعمرة/غم لحم للوجبة الاولى والثانية على التوالي. سجلت الصدور مدى ١٤,٣٨،١١,٩٠ و ١٠×٠,٥٨،١,١٧^٤ وحدة تكوين مستعمرة/غم لحم لكل من العلامتين التجاريتين **Seara** و **Sadia** على التوالي. اما مدى عدد البكتريا الآلفة للبرودة فقد كان ١,٩٥-١٣,٧٤ و ٠,٩٠- ٢٧,٤٢×١٠^٤وحدة تكوين مستعمرة/غم لحم في الذبائح بينما في الافخاذ فكانت ٠,٥٥-٨,٥٦ و ٠,٣٦-٨,٠٠×١٠^٤وحدة تكوين مستعمرة /غم لحم وفي الصدور كانت ٠,٧٣-٨,٨٨ و ١,١٤- ٩,٣٦ ×١٠^٤وحدة تكوين مستعمرة/غم لحم. ان عدد البكتريا المتحملة للبرودة للذبائح وللوجبتين الاولى والثانية فكانت بمدى ١,٩٦-١٤,٢٨ و ١,٢٨-٢١,٨٠ ×١٠^٤وحدة تكوين مستعمرة/غم لحم . والافخاذ سجلت مدى ٠,٦٤-١٢,٦٤ و ٠,٤٨- ٧,٦٨ ×١٠^٤ وحدة تكوين المستعمرة/غم لحم وللوجبتين الاولى والثانية على التوالي. والصدور سجلت مدى ٠,٧٧- ١٣,١٤ و ١,١٧- ٨,٤٤ ×١٠^٤وحدة تكوين مستعمرة/غم لحم وللوجبتين الاولى والثانية على التوالي. العدد الاكثر احتمالا للبكتريا القولونيه لكل العينات ولكلا الوجبتين اقل من ١وحدة تكوين مستعمرة/غم لحم. وكذلك خلت كل العينات ولكل العلامات وفي كلا الوجبتين من بكتريا *E.coli* O157:H7 و بكتريا Coagulase positive *Staphylococcus aureus* وانواع بكتريا *Salmonella*.

GENETIC VARIABILITY AND GENOTYPIC, PHENOTYPIC CORRELATION STUDIES FOR YIELD AND RELATED TRAITS IN SINGLE CROSS HYBRIDS OF MAIZE (*Zea mays L.*)

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Abstract

The study was connected to evaluate seven mays hybrids for genetic variability for yield components at Faculty of Agriculture and forestry field university of Duhok during spring 2011 and 2012 .

The result show highly significant different between the genotype for plant height, leaf area , and significant at 0.05 level for ear height ,number. of grain ear^{-1} and non-significant for 300-grain weight and grain yield plant^{-1} in spring 2011. While the main square for genotype was highly significant for leaf area and significant for plant height, ear height ,number of row ear-^{1},and non-significant for rest characters ,also show significant effect between hybrid and yield. Plant height exhibited significant positive genotypic correlation with ear height, leaf area ,300-grain weight ,and not significant with yield plant^{-1} while the phenotypic correlation show appositive non significant correlation between number of row with leaf area with (rph =0.60).

KEYWORDS*: Hybrids ,Yield ,Phenotypic ,Genotypic , Variability*

Introduction

The use of hybrids was most important advance in the cultivation of maize. The first commercial sale of hybrid seed started in 1984.Inbred lines of Maize show general deterioration in yield and vigor, but hybridization between two inbred immediately and completely recovers. In many cases their yield exceeds that of the varieties from which inbred were derived (shull,1908).Increased production per unit area is the primary objective in maize breeding programs and grain yield is the most important and complex character with the maize breeders work which inherited trait being quantitatively controlled by numerous minor genes , as a result of different vital processes of plants, such as photosynthesis ,transpiration and storage of food materials , maize display an orderly sequence of development o n yield components number of ear plant^{-1},number of kernels row ,number of rows in ear and kernel weight (Viola *et al.*, 2003).Genetic improvement in traits of economic importance along with maintaining sufficient amount of variability is always the desirable objective in maize breeding programs (Hallauer and Scobs,1973).

The variability is a key to crop improvement (Welsh,1981).Grzesiak (2001) observed considerable genotypes variability among various maize genotypes for different traits. .Ihsan *etal*., (2005) also reported significant genetic differences for morphological parameter for maize genotypes .

Large amount of genetic variability has been observed to occur in the original accessions and races among sampled population representing different climatic and geographical regions (Ilarsalan *et al*.,2002),(Abayi *et al*.,*(2004)*, (Naushad *et al*.,2007), and Melil *et al*.,(2013) Observed significant genetic variation in important agronomic traits. Selection for specific trait is known to result in correlated response in certain other traits.

Improvement yield is the most important target trait in most of crops.AL-Ahmad, (2004), Aydin *et al*.,(2007) and Najeeb *et al*.,(2009) indicated that the correlation value were positive and significant between grain yield and each of ear diameter ,ear length and number of kernels row^{-1}. Brar *et a*l.,(2008) denoted that studies conducted on (15) single cross hybrid (F1) maize over two locations revealed ear length and number of rows^{-1} ear had significant positive correlation with yield .

The present study was conducted to evaluate the performance of different maize hybrids and genotypic and phenotypic correlation between tries under agroclimatic condition of Duhok ,Kurdistan Region -Iraq.

Materials and methods

The present study was conducted to evaluate seven maize hybrids for genetic variability(IK8 ×IK58 ,ZP707× UN44052 ,IK8 × HS ,IK8 × DK ,OH40 × ZP-301 ,IK8 × OH40, UN44052× DK) in yield and yield components at Faculty of

Agriculture and Forestry field university of Duhok, during spring 15/3/2011 and 2012.

These hybrids were selected from maize programs which were applied in the field crops department of Faculty of Agriculture and were having more heterogeneity as compared to the synthetic.

The experiment was laid out in randomized complete block design with three replications.

The genotypes were grown in two rows plots, with row length of 3m having row to row and plant to plant distance of 0.75 and 0.25 m respectively .Two seeds per hole were planted, which were thinned to one plant per hole at 4-5 leaf stage. Compound fertilizer ,NPK Russian origin, 17,17,17 was added with quantity 600kg/ha after plowing and urea 46% N once with quantity 200kg /ha at the beginning of flowering .Standard cultural practices were followed from sowing till harvesting during the entire crop season .

The data recorded on the basis of the average experimental unit (five plant per experimental unit) ,for yield related traits viz, number of grain row^{-1} number of row per ear^{-1} , 300-grain weight and grain yield plant^{-1}.Data were statistically analysis using analysis of variance appropriate for randomize complete block design. Mean were compared using Multiple Range Test at 0.01 and 0.05 level of probability (steel and Torric 1984).

Result and discussion

The analysis of variance (Table 1) showed the mean square of genotypes was highly significant for plant height, leaf area and significant at 0.05 level for ear height, Nu. of rows/ear $^{-1}$,Nu .of grain ear^{-1} and non-significant for 300 grain weight and grain yield plant^{-1} in spring 2011,while the mean square for genotype was high significant for leaf area and significant for plant height, ear height, Nu. of row ear^{-1} and non-significant for the other characters in spring 2012 .

Table (1): The analysis of variance for yield and some studied characters.

s.o.v	d.f	year	M.S						
			Plant height (cm)	Ear height (cm)	Leaf area (Cm^2)	Nu.of rows ear^{-1}	Nu.of grains row^{-1}	300grain weight (g)	grain yield plant^{-1} (g)
Replicates	2	2011	2.88	2.64	4568.47	6.04	4.33	214.31	824.03
Hybrids	6		308.72**	137.72 *	33681.15 **	6.63*	23.93	53.71	795.33
Error	12		3.03	1.20	733.81	2.82	5.38	100.73	344.55
Replicates	2	2012	60.33	9.47	2651.80	3.04	10.90	81.11	1114.28
Hybrids	6		193.99 *	163.02*	19795.91**	3.74	22.52	14.64	207.79
Error	12		77.51	42.22	876.27	1.93	20.23	16.68	260.37

Table (2) showed combing analysis results of genotypes across for seven characters of maize. The mean square of genotypes was height significant for plant height, leaf area and significant for the other characters .The mean square of year was significant at 0.01 level for all studied characters except grain yield plant^{-1} which was significant at 0.05 level while Number of rows ear^{-1}was not significant. The Years x Hybrids interaction was significant for all the studied characters except Nu.of rows ear^{1}.

Table (2): combing analysis for two years and some characters for maize varieties .

S.O.V	d.f	Plant height (cm)	Ear height (cm)	Leaf area (cm^2)	Nu.of $row ear^{-1}$	Nu.of grains row^{-1}	300grain weight (g)	grain yield $plant^{-1}$ (g)
Years	1	2608.03**	1167.88 **	150335.56**	0.20	168.02 **	514.80 **	291.97 *
Repl.X years	4	65.37	29.25	8882.68 **	4.79 *	14.45 *	83.08 *	525.10 *
Hybrids	5	286.71 **	95.42 *	25967.08 **	4.89 *	22.13 *	18.24	398.11 *
Years X Hybrids	5	98.23 *	100.53 *	2866.45 *	2.24	17.86 *	65.50 *	961.23 *
Error	5	36.79	17.75	1177.50	1.34	3.76	22.04	71.24

Table (3) confirmed that the effect of years on grain yield and its components were significant in all characters with the exception of nu. of rows ear^{-1} .Results indicated the exceeding of the year 2012 in all studied characters compared to year 2011 by 165.9 ,78.35 , 669.90 , 39.30 ,51.50 ,86.95 for the characters plant height ,ear height , Leaf area ,Nu.of grain row^{-1} ,300 grain weight and grain yield $plant^{-1}$,respectively.

Table(3): effect of years in grain yield ,its components and grown characters.

character years	Plant height (cm)	Ear height (cm)	Leaf area (cm^2)	Nu.of $row ear^{-1}$	Nu.of $grains row^{-1}$	300grain weight (g)	grain yield $plant^{-1}$ (g)
2011	143.63 b	63.41 b	500.49 a	16.00 a	33.63 b	61.65 a	79.48 a
2012	165.95 a	78.35 a	669.90 a	15.80 a	39. 30 a	51.50 a	86.95 a

Data in table (4) indicated that there is significant difference between hybrids in all studied characters.

The results of the effect of genotypes on plant height revealed significant differences between genotypes, and the maximum plant height record by the hybrid (IK8 x HS) (170.13 cm) ,while the minimum plant height recorded by hybrid (IK8 x IK58) (142.33 cm). For ear height the genotypes (IK8 x DK) surpassed others genotypes (75.50 cm), followed by the (ZP707 x UN44052) which gave (74,36 cm) but the genotype (IK8 x IK58) was inferior (62.00 cm).

The data in the same table indicated the maximum Leaf area value recorded by the hybrid (OH40 x ZP-301) market with 733.99cm^2 and followed by the hybrid (IK8 x DK)with 646.53 cm^2, while the minimum value exhibited by the hybrid (IK8 x HS) which was 573.73 cm^2.

From the same table, the hybrid (IK8 x OH40) showed the highest number of rows ear^{-1} which reached 18.00 rows. Minimum value recorded by hybrid (IK8 x IK58) (13.33 rows) The predominating of hybrid (IK8 x OH40) compared to hybrid (IK8 x IK58) was recorded 25 % .

Concerning effect of hybrid on Nu. of grains row^{-1} ,was also influenced significantly, (table 4) showed that the hybrid (ZP707 xUN44052) gave the highest Nu. of grains row^{-1} (40.33) , but the least value was (33.00) in hybrid (IK8 xHS).For the 300 grain weight ,no significant difference were noticed between all hybrids and the value rang between 55.00 to 59.79 for hybrid (IK8 x IK58) and hybrid (ZP707 x UN44052) respectively.

The data in table (4) showed that there is no significant differences observed between the hybrids in plant yield except the hybrid (IK8 x IK58)which record 60.96g where compared with the hybrid (IK8 x SH) (91.72) . This finding are accordance with; Najeeb *etal*.(2009).Brar *et al*.(2008). and Aydin *et al*.(2007)

Table (4): Effect of genotypes on studied characters.

variety	Plant height (cm)	Ear height (cm)	Leaf area (cm^2)	Nu.of rows ear^{-1}	Nu.of grains row^{-1}	300grain weight (g)	grain yield $plant^{-1}$ (g)
IK8 x IK58	142.33 c	62.00 c	536.41 cd	13.33 c	39.00 ab	55.00 a	60..96 b
Zp707xUN44052	158.16 ab	74.36 ab	571.76 c	16.33 ab	40.33 a	59.79 a	85.80 a
IK8 x HS	170.13 a	75.33 a	573.76 c	15.33 bc	33.00 d	59.70 a	91.72 a
IK8 x DK	158.66 ab	75.50 a	646.53 b	16.00 ab	34.66 cd	53.60 a	79.56 a
OH40 x ZP-301	150.43 bc	69.10 abc	733.99 a	16.66 ab	35.33 bcd	58.42 a	90.56 a
IK8 x OH40	148.73 bc	68.33 bc	527,79 cd	18.00 a	38.00 abc	55.40 a	84.96 a
UN44052 x DK	151.36 bc	72.66 ab	477.93 d	15.66 abc	34.00 cd	55.16 a	87.66 a

Table (5) showed significant effect of interaction between hybrids and years .The maximum plant height value recorded by the interaction of hybrid(IK8 x HS) with the year2 was 182.50 cm, and followed by 171.00cm exhibited by the interaction between hybrid (UN44052 x DK) with the same year, while the lowest plant height value recorded by the association between hybrid (IK8 x IK58) with year1 was 128.50 cm . Regarding effect the interaction between hybrids and years ,maximum ear height exhibited by hybrid (IK8 x HS)with the year 2 was 90.50cm and the minimum value was52.50 cm ,by the interaction hybrid (IK8 x IK58) with the year1.Fore the Leaf area the maximum value which was 819.00 cm^2 by the interaction (OH40xZP-301)xY2 which the lowest value exhibited by the interaction(ZP707xUN44052) xY1 with 385.91 cm^2.The same table showed significant differences effect of interaction between genotypes and years in Nu. of rows ear^{-1} ,the interaction between (OH40xZP-301)xY1 and (IK8xOH40) xY1 recorded the maximum Nu.of rows ear^{-1} which were 18.00 rows and the lowest value was 13.00 rows by the interaction between (IK8xIK58)xY1 .From the Nu.of grains row^{-1} ,the interaction between(IK8xIK58)xY2 gave maximum Nu.,of grains row^{-1} while the lowest Nu.of grains row^{-1} was 30.00 exhibited by the interaction between (UN44052xDX) xY1 . Regarding the 300 grain weight ,the differences of the interaction effect on the 300 grain weight ,the highest value was 75.23 g. at the interaction between (ZP707xUN44052)xY1 ,while the lowest value recorded by the interaction (IK8xIK58)xY2 which was 44.64g.The results were confirms in (table 5),where the interaction was significant the treatment (IK8 xOH40) xY1 have highest grain yield $plant^{-1}$ with (113.46 g) followed by (IK8 x HS)xY1 with 104.10 g ,while the treatment (ZP707xUN44052)xY1 gave the lowest value with 51.72 g .This result agreement with Grzesiak(2001); Naushad *et al*.,(2007); Ihsan et al.(2005); and Al.Ahmad *et al*.(2004) .

Table(5): effect of interaction between genotypes and years on studied characters.

Years	variety	Plant height (cm)	Ear height (cm)	Leaf area (cm^2)	Nu.of rowsear^{-1}	Nu.of grains row^{-1}	300grain weight (g)	grain yield plant^{-1} (g)
2011 (Y1)	IK8 x IK58	128.50 f	52.50 f	444.62 fg	13.00 c	34.00 def	60.00 abcde	52.86 d
	ZP707 x UN44052	135.00 ef	55.60 ef	385.91 g	17.00 ab	36.00 cdef	75.23 a	51.72 d
	IK8 x HS	163.95 abc	67.75 cde	519.72 ef	16.00 abc	32.50 ef	69.24 ab	104.10 ab
	IK8 x DK	144.50 cdef	71.50 bcd	525.85 ef	16.00 abc	34.00 def	55.70 bcde	61.97 cd
	OH40 x ZP-301	144.40 cdef	67.65 cde	691.49 bc	18.00 a	33.00 ef	56.50 bcde	85.84 abc
	IK8 x OH40	143.70 def	65.00 def	418.21 fg	18.00 a	40.00 bcd	64.44 abc	113.46 a
	UN44052 x DK	141.55 ef	64.85 def	431.89 fg	15.50 abc	30.00 f	59.28 abcde	80.78 bc
2012 (Y2)	IK8 x IK58	170.00 ab	81.00abc	720.00 ab	14.00 bc	48.00 a	44.64 e	77.17 bcd
	ZP707 x UN44052	169.75 ab	83.75 ab	664.69 bcd	16.00 abc	42.00 ab	52.08 cde	102.84 ab
	IK8 x HS	182.50 a	90.50 a	681.75 bcd	14.00 bc	34.00 def	50.16 cde	66.99 cd
	IK8 x DK	165.75 ab	77.50 abcd	706.88 b	16.00 abc	35.00 def	52.56 cde	88.36 abc
	OH40 x ZP-301	162.50 bcd	72.00 bcd	819.00 a	14.00 bc	40.00 bcd	62.28 abcd	100.03 ab
	IK8 x OH40	151.25 bcde	65.50 def	582.58 bcd	18.00 a	37.00 bcde	50.89 cde	70.71 cd
	UN44052 x DK	171.00 ab	86.50 a	570.00 de	16.00 abc	42.00 abc	46.92 de	101.44 ab

Plant height exhibited significant positive genotypic correlation with ear height rG=0.67(Table 6).

However the genotypic correlation of plant height with Leaf area, 300 kernel weight and yield was not significant. Plant height showed significant positive phenotypic correlation with ear height (rph= o.65) and non significant with other traits .positive and significant genotypic between ear height with leaf area and kernel yield plant^{-1} with (rG= 0.55 and rG =60) respectively.

The same result obtained in case of phenotypic correlation between ear height and leaf area, rph = 0.53. The other traits showed positive or negative and non-significant genotypic between them

Table (6) Genotypic (Above Diagonal and phenotypic Bellow Diagonal) correlation among various traits of seven maize hybrid-2011.

	Plant height(cm)	Ear Height (cm)	Leaf area (cm^2)	Nu.of rows ear^{-1}	Nu.of grains row^{-1}	300grain weight (g)	grain yieldplant^{-1} (g)
Plant height(cm)		0.67**	0.33	0.32	0.05	0.33	0.50
Ear height (cm)	0.65*		0.55*	0.26	0.21	0.19	0.60*
Leaf area (cm^2)	0.29	0.53*		0.11	-0.07	0.18	0.35
Nu.of row ear^{-1}	0. 19	0.11	0.12		0.01	-0.07	0.13
Nu.of grains row-1	0.03	0.14	-0.12	-0.05		0.12	0.45
300grain weight(g)	0.15	0.09	0.06	-0.16	0.24		0.29
grain yield plant^{-1} (g)	0.35	0.39	0.13	-0.005	0.37	0.27	

rG = genotypic correlation
rph = phenotypic correlation

Table(7): Genotypic(above Diagonal and phenotypic bellow Diagonal) correlation among various traits of seven maize hybrid -2012.

	Plant height (cm)	Ear height (cm)	Leaf area (cm^2)	Nu.of $rowsear^{-1}$	Nu.of grains row^{-1}	300grain weight (g)	grain $yieldplant^{-1}$ (g)
Plant height(cm)		0.56*	0.10	-0.37	0.09	-0.13	0.20
Ear height (cm)	0.43		0.03	-0.39	0.04	-0.01	0.12
Leaf area (cm^2)	0.03	-0.05		-0.73**	-0.17	0.39	0.23
Nu.of row ear^{-1}	-0.22	-0.24	-0.60*		-0.10	-0.30	-0.002
Nu.ofgrains row^{-1}	-0.06	0.00	0.01	-0.41		-0.37	0.33
300grain weight(g)	0.08	0.01	0.27	-0.20	-0.94		0.08
grain yield $plant^{-1}$ (g)	0.09	0.14	0.20	-0.13	0.11	0.40	

The data in table (7) showed the genotypic and phenotypic correlation between the many traits .The plant height exhibited a positive genotypic correlation with ear height and positive or negative genotypic correlation and significant with the other traits .Leaf area showed negative genotypic correlation with no .of row ear^{-1} with (rG=0.73) while the phonotypic correlation showed a positive and significant correlation between Nu. of row ear^{-1} with Leaf area with (rph=0.60) ,but the remaining traits showed positive and negative phenotypic correlation between them.

Conclusion

preceding results and discussion revealed that different maize hybrids have performed differently for yield and yield component may be concluded that maize and have resulted in better performance for yield and yield components, and are recommended for commercial cultivation in Duhok and other location having the similar climatic condition, after development of improved production technology, and also it concluded that selection for genotypes having high yield potential may be based on plant height and Nu. of row ear^{-1}.

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الخلاصة:

اجريت هذه الدراسه لتقويم التباين الوراثي للحاصل ومكوناته لسبعة هجن من الذره الصفراء للموسمي الربيعي ٢٠١١ و٢٠١٢ في حقل كلية الزراعه والغابات جامعة دهوك .

أظهرت النتائج وجود فروقات عاليه المعنوية بين التراكيب الوراثيه لأرتفاع النبات والمساحه الورقيه في حين كانت الفروقات على مستوى ٠،٠٥لأرتفاع النبات وعدد البذور للعرنوص غير معنويه لصفة وزن ٣٠٠ حبه وحاصل النبات للموسم الربيعي ٢٠١١.بنما كانت الفروقات عالية المعنويه للمساحه الورقيه ومعنويه لأرتفاع العرنوص وغير معنوي لبقية الصفات ،كما أظهرت النتائج وجود علاقة إرتباط بين إرتفاع بين النبات وإرتفاع العرنوص والمساحه الورقيه ووزن ٣٠٠ حبه وغير معنويه مع حاصل النبات ،كما أظهرت إرتباط المظهري إرتباط موجب وغير معنوي مع عدد الصفوف للعرنوص الواحد والمساحه الورقيه ، بينما كان ألأرتباط موجبآ وغير معنويه بين عدد الصفوف للعرنوص و المساحه الورقيه بقيمة ٦٠،٠ .

EFFECT OF DESIGN PARAMETERS ON THE SEPARATION EFFICIENCY OF VERTICAL GAS/LIQUID SEPARATOR IN OIL GAS PIPELINE

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Abstract

The main objective of this work is to size a vertical axis gas liquid separator to separate gas and liquid on a long distance in gas-liquid pipelines. This is necessary to ensure the safe operation of the compressors used along the pipelines.

The separator equipped with wave plate demister (vane pack) as the secondary separator.

The design is achieved through investigation of the parameters which has an effect on the separation efficiency of the separator. In this study, inlet pipe diameter, vessel bend spacing, vane pack bend angle, momentum breaker, vane pack plate spacing, and the number of bends in pack were used. From the results of this study overall separation efficiency of 99.99% has been achieved.

KEYWORDS: *phase separation, efficiency, vertical separator, vane pack.*

1. Introduction

Phase separators, particularly gas / liquid two-phase separators, are employed in a very wide range of industries. They can range from the small size application to large vessel employ on offshore oil production platforms or slug catchers at the shore terminals of undersea transmission pipelines.

Phase separator separates the well fluids into gas and liquid. A two-phase separator can be horizontal, vertical or spherical. The liquid (oil, emulsion) leaves the vessel at the bottom through a level-control or dump valve. The gas leaves the vessel at the top, passing through a mist extractor to remove the small liquid droplets in the gas. The mechanisms of phase separation employed include setting under the action of gravity or centrifugal forces and impaction onto solid surfaces.

1.1. Separator Type

There is primary (gravity) separator (vertical or horizontal) and secondary separator or demister employed for phase separation. The sizes of drop which can be removed from the gas phase can vary over a wide range in demister, depending on their form of source. According to their size, it can be classified as a rough approximation; those above 10 μ m are referred to as sprays, below these range droplets are regarded as mists or aerosols. Sprays and mists are not uniform but always consist of drops of a distribution of sizes.

The type of demister used will rely on the drop size distribution of the mist and the efficiency required. A combination of types will be employed in many cases. Gravity separator or cyclones are used if the mist with drops above 100 μm a unit capable of handling finer drops followed should be necessary. Therefore, for finer drops, whether the main part of the mist or the tail of a coarser mist, wave plate or mesh pad demisters or axial flow cyclones are suggested.

Wave plate demisters or separators (also known as vane pack demisters) are composed of series of wave plates laid side by side. The inability of drops to follow the gas through the plates can determine the effectiveness of wave plate. The drops tend to follow a straighter line, impinge on the wall and drain away. Additional features are employed in the form of recessed of protruding channels to assist drainage of the liquid, shown in Figure (1).

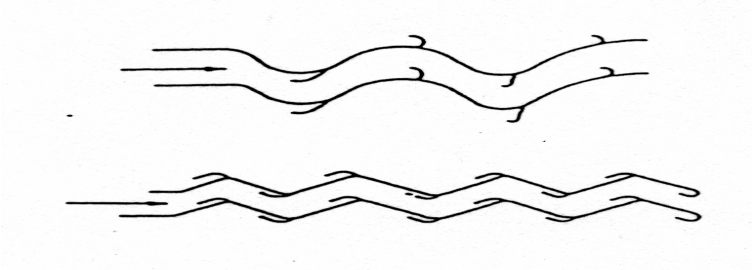

Figure (1) arrangement of typical wave plate

The wave plate units are operated with vertical flow or horizontal flow. In the case of vertical flow the collected liquid has drain counter-currently to the gas flow. Consequently, there is a limitation that is counter-current flow limitation or flooding when the gas begins to hold liquid up with it. But for horizontal gas flow, liquid drainage is obviously perpendicular to the gas flow. In both cases local re-entrainment and loss of efficiency can be cause by the accumulation of liquid.

The bend angle, the plate spacing and the approach velocity of the gas are the important parameters of the wave plate geometry. Excepting grade efficiency, pressure drop through the demister is also important parameter.

The main problem of drop removal is related to the fact that the effectiveness of many separators is decisively affected by the individual drop size. For a given configuration of demister, the fractional separation efficiency represents the probability of a drop with a given size being retained in the separator. The curve obtained by plotting the fractional separation efficiency as a function of particle size is called fractional separation curve. This can be combined with the particle size distribution to give the effectiveness of a specific separator with a particular laden gas flow. If a given contaminant is to be fully separated, the entire fractional separation curve must lie on the left of the oversize cumulative distribution curve. On the other hand, if the fractional separation curve lies on the right of the oversize cumulative distribution curve, no separation occurs at all, i.e. the separator is totally ineffective. Frequently the two curves overlap, and partial separation takes place Figure (2). The overall separation efficiency η_O is calculated from the fractional separation efficiency η_F:

$$\eta_O = \int_{d_{\min}}^{d_{\max}} \eta_F \, v(d) \, dd \quad (1)$$

The integration is performed from the smallest to the largest particle diameter. However, for the practical purposes, it is sufficient if the particle size distribution and the fractional separation efficiency are available in graphical form. The approximate overall separation efficiency is then calculated from the following equation:

$$\eta_O = \sum \eta_F \, v_i \quad (2)$$

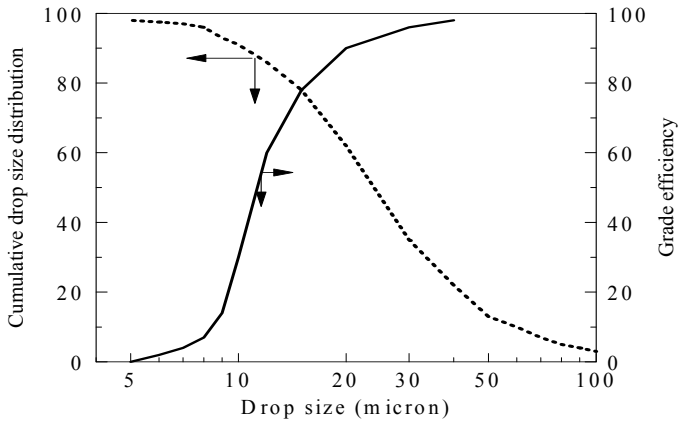

Figure (2) Particle size distribution curve and fractional separation efficiency curve

From the above it can be seen that sufficiently large drops are always completely separated while small drops can escape separation altogether. However, what the meaning of large and small is depends on the particular piece of equipment.

Typical grade efficiency curves for wave plates, measured by Azzopardi *et al.* (2000) are shown in Figure (3) where the effect of gas velocity can be seen. As well as data from plain wave plates, the figure also shows data from a unit with drainage channels or hooks. These results were taken on a carefully designed wind tunnel, which provided (nearly) saturated air to minimize evaporation of the drops. The drop flow was sampled before and after the demister and sorted into sizes. From this the grade efficiency could be determined.

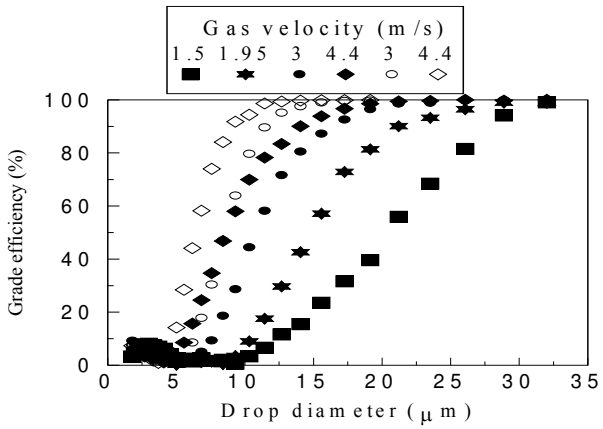

Figure (3) Effect of gas velocity and wave plate geometry on grade efficiency for wave plate demisters.

The occurrence of re-entrainment in wave plate demisters has been studied by Houghton and Radford (1939), Monat et al. (1986), Verlaan (1991) and Sanaullah and Azzopardi (1999). For vertical wave plates (gas up flow, downwards drainage of the liquid) the mechanism for the decrease in efficiency has been attributed to flooding of the draining film by the upward shear of the gas, Verlaan (1991). Flooding is the condition at which an upwards gas flow starts to prevent the down flow of liquid. From their experiments with air/water at ambient conditions, Monat *et al.* (1986) proposed that re-entrainment would occur when a critical value of dimensionless re-entrainment number ($U_g^4\rho_g^2/\rho_l g\sigma$) was exceeded. This group is the fourth power of the dimensionless velocity, usually called the Kutateladze number, a parameter much used in the analysis of flooding processes. For horizontal systems Sanaullah and Azzopardi (1999) propose that it is the centrifugal forces on the film as it goes around a corner that causes re-entrainment. They propose a simple model which successfully describes the limiting gas flow determined experimentally.

1.2. Pressure Drop

Pressure drop across the unit is another important design parameter. A correlation has been proposed by Wilkinson (1999) which predicts a large bank of data from plain wave plates to within +26/-37% with all data being encompassed by ~±53%. More interestingly, Burkholz (1989) has proposed that pressure drop correlates well with the drop diameter for which the collection efficiency is 50%. Figure (4) show that data from both plain wave plates and those with drainage channels both lie on one line when plotted in this way.

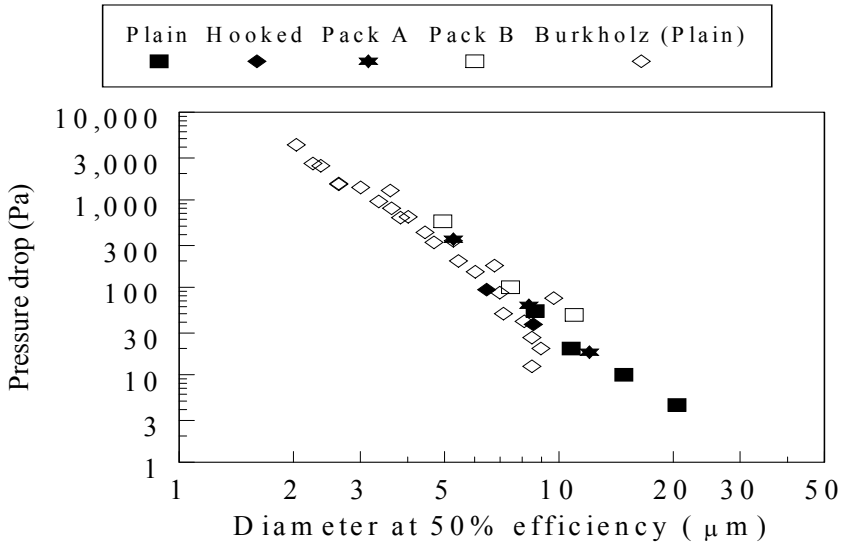

Figure (4) correlation of pressure drop with drop size whose collection efficiency is 50%.

1.3. Diameter and Height of Vessel:

Sinnott (1999) suggested that the settling velocity of the liquid droplets for the design of separation vessels can be estimated by the equation (3) given below:

$$Ut = k \cdot \left(\frac{\rho_l - \rho_g}{\rho_g} \right)^{0.5} \qquad (3)$$

Where U_t= settling velocity (m/s)
k = 0. 07
ρl = liquid density (kg/m^3)
ρg = gas density (kg/m^3)

The diameter of the vessel must be large enough to show the gas down to below the velocity at which the particles will settle out.

Therefore, the minimum allowable diameter can be calculated by the following equation:

$D_v = \{4V_v/\pi\, U_t\}^{0.5}$ (4)

Where D_v= minimum vessel diameter, m
V_v=gas volumetric flow rate, m^3/s

The height of the vessel can be found from the optimum length to diameter ratio as a summarized in table (1):

Table(1) the range of optimum L/D ratio for vertical separator

Operating pressure range(barg)	Optimum L/
Atmospheric pressure to 17 barg	≤ 3
18 barg to 34 barg	≤ 4
Higher than 35 barg	≤ 5

2. Experimental Facility

A schematic of a vertical separator is shown in Figure (5) the inlet flow enters the vessel through the side. The inlet diverter does the initial gross separation. Liquid flows down to the liquid collection section of the vessel, then go down to the liquid outlet. When liquid reaches equilibrium, gas bubbles flow counter to the direction of liquid flow and eventually migrate to the vapor space. Then liquid leaves the vessel through the liquid dump valve, which is regulated by a level controller. The level controller senses changes in liquid level and controls the dump valve accordingly.

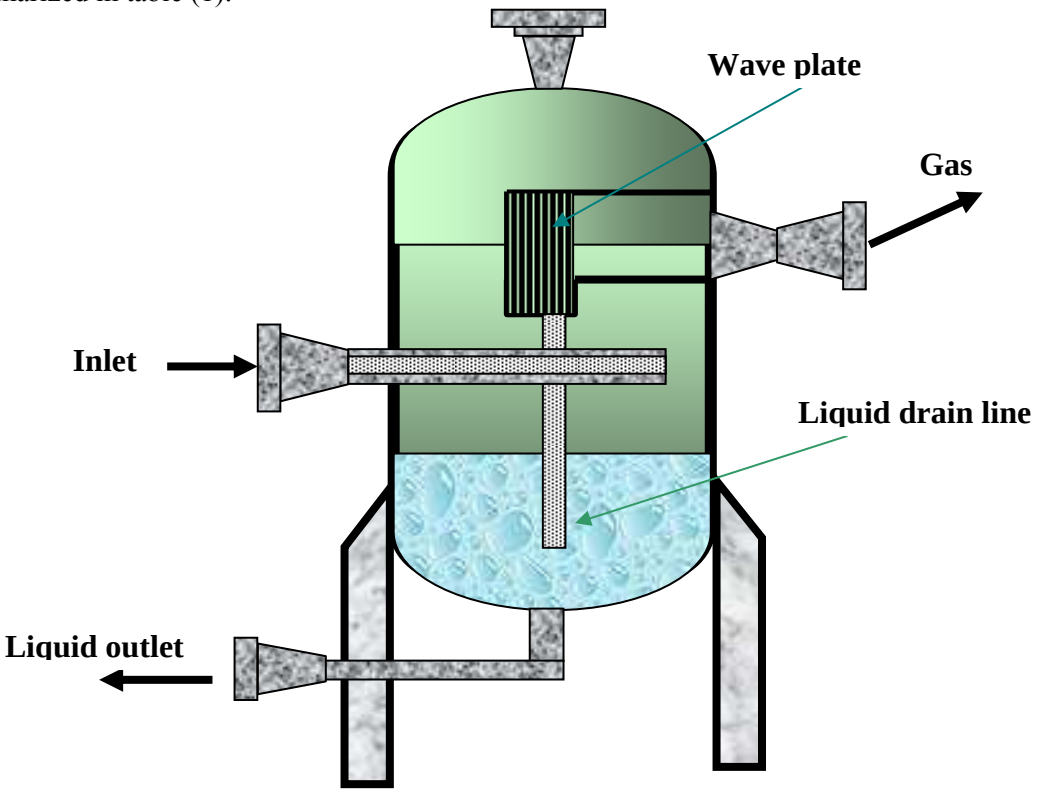

Figure (5) Vertical separator for gas/liquid separation

Gas flows over the inlet diverter and then up toward the gas outlet. In the gravity setting section, the liquid drops fall downward, counter to gas flow. Gas goes through the wave plate demisters before leaving the vessel. A controller opens and closes the pressure control valve at the gas outlet to maintain the desired vessel pressure.

3. Process Conditions:

For this study, the feed into the vertical separator is given in Table (2):

Table (2) process conditions

Gas flow ,Q (MMscf/D)	**150**
Gas to liquid ratio (bbl/scf)	30000
Liquid flow ,L(bbl/day)	4500000
Operating pressure ,P(bar)	20
Operating temperature ,T°	25

From the given conditions, the properties of gas and liquid as well as their mass flow rates are given in Table (3):

Table (3) properties of gas and liquid

Gas mass flow rate , Mv (t/h)	**119.85**
Liquid mass flow rate ,Ml(t/h)	16.77
Gas density, ρv(kg/m3)	13.52
Liquid density, ρl(kg/m3)	550.47
Gas viscosity, μv(kg/ms)	$1.161*10^{-5}$
Liquid viscosity, μv(kg/ms)	$1.668*10^{-4}$
Surface tension ,σ(N/m)	0.00994

4. Results and Discussion

4.1. Inlet pipe diameter

To obtain optimum the pipe diameter the inlet pipe diameter is changed according to the values of the standard pipe sizes.

The effects of the change in pipe diameter on the overall separator efficiency are shown in Figure (6). From figure, it can be seen that as the pipe diameter increases the overall efficiency decreases initially until a diameter of 0.2 m is reached. This can be explained by dispersion of larger droplets into smaller droplets due to increase in the vapour velocity .higher velocity tends to break large droplets into smaller droplets. At the same time, using larger pipe size would have an effect of producing larger droplets which should be relatively easier to coalesce and removed upon accumulation compare to smaller droplets. As the pipe diameter increases beyond 0.2 m, the overall efficiency increases to a value of 99.99%. For the pipe diameter of 1.067m .from the figure, it is also observed that the overall efficiency only increases by small amount of 0.047 when the pipe diameter is increased from 0.813m to 1.067m, therefore the pipe diameter of 0.831 m is chosen as the optimum diameter.

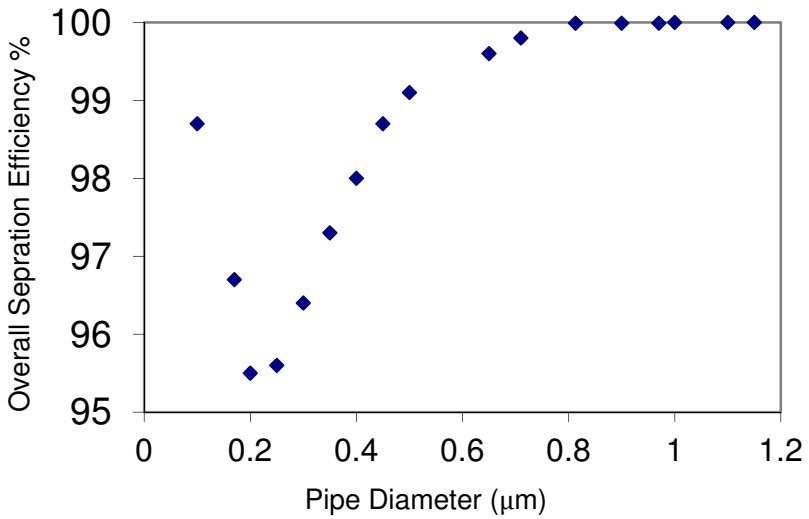

Figure (6) effect of varying inlet pipe diameter on the overall separator efficiency

4.2. Vessel Bend Spacing

With inlet pipe diameter fixed at 0.813 and other parameters, the vessel bend spacing is changed at values of 0.3, 0.4 and 0.5m. Figure (7) shows the effect of varying the spacing on the adjusted bend efficiency. From the figure, the efficiency achieved is almost the same for droplet sizes less than 20 μm and only at higher droplet sizes the efficiency greater at a spacing of 0.3m. Therefore the spacing of 0.3 m is chosen as the optimum spacing.

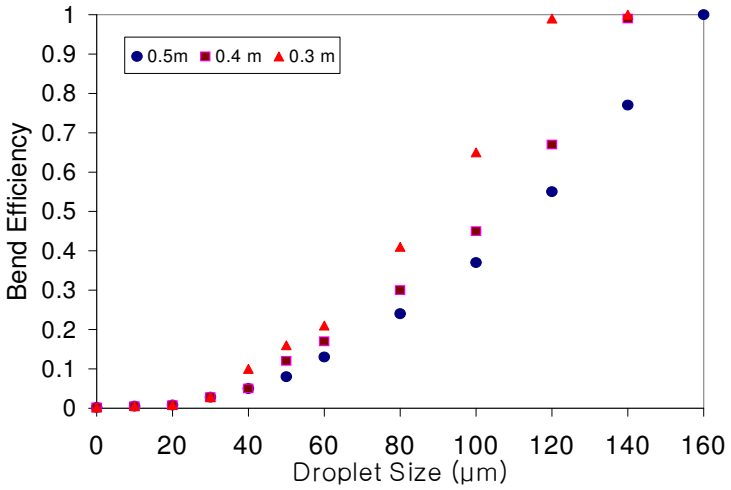

Figure (7) effects of varying vessel bend spacing on the adjusted bend efficiency.

4.3. Momentum Breaker Bend Angle

With fixing all parameters. The effect of the change in momentum breaker efficiency is investigated when angle is changed to 120°, 150° and 180°. The variation is shown in Figure (8). From the figure, as can be seen, the efficiency achieved is almost the same for droplet sizes less than 20 µm and then efficiency started to increase with increasing droplets diameter. In addition, it is clear from figure at higher droplet sizes the efficiency greater at angle 180° degree.

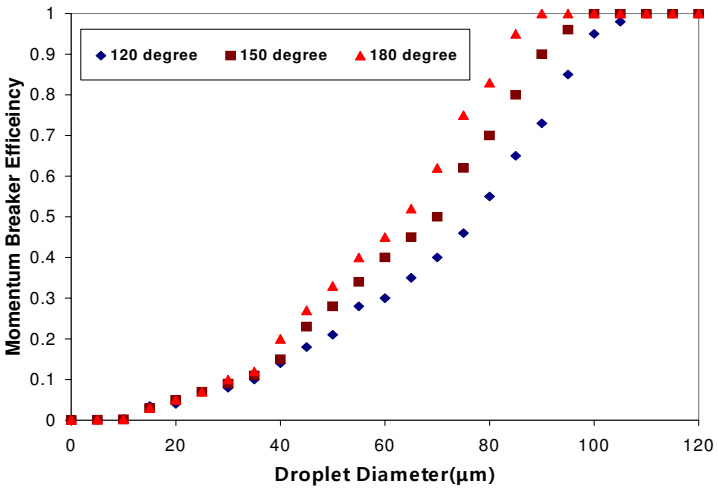

Figure (8) effect of varying momentum breaker bend angle on the adjusted bend efficiency

4.4. Vane Plate Spacing

From Figure (9), it is clear that the overall vane efficiency increases as the plate spacing is reduced. The figure also shows that changes in the vane plate spacing have more pronounced effect on the efficiency for droplet diameters less than 10 m compared to the parameters which were changed previously. In addition, reducing the vane plate spacing will lead to higher effectiveness in removing smaller droplets. Reducing the plate spacing will lead to droplets to collide at the bend wall at higher velocity and trap in the drainage channel. The pressure drops as estimated from the correlation shown in figure1 are negligible for all three spacing as they are all less than 5 pa. Therefore the plate spacing of 0.06m is chosen as the optimum plate spacing.

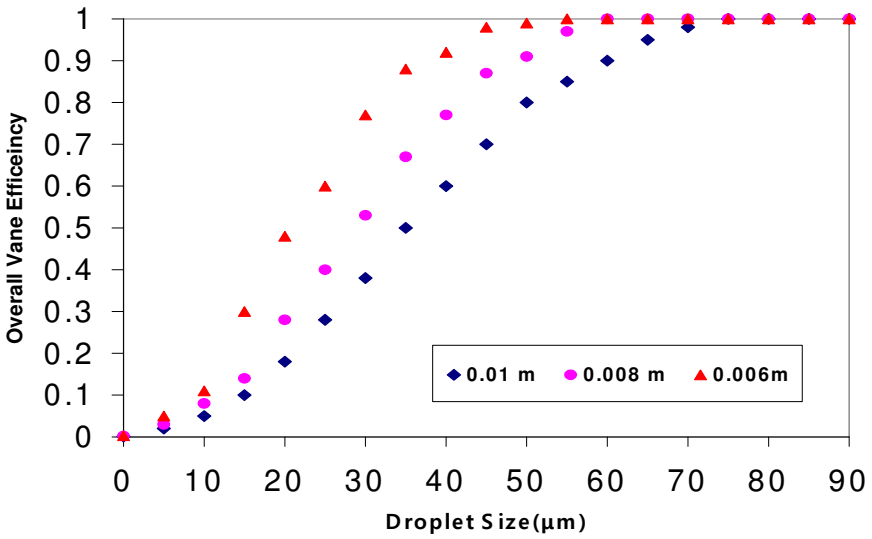

Figure (9) effect of varying the vane plate spacing on the overall vane efficiency

4.5. Number of Bends in Vane Pack

It was found from figure (10) that the separation efficiency increases as the number of bend in the vane increases. This is fairly obvious as the droplets have a higher chance of collide at the bend wall and coalesce to from droplets. From figure it is also observed that a certain vane has certain limitation on the size of droplets that it can separate, which does not vary with change in the number of bend in the vane .therefore increasing the bend number further will not help in achieving a better separation in terms of removal of more smaller droplets, but will help in more complete separation of larger droplets..

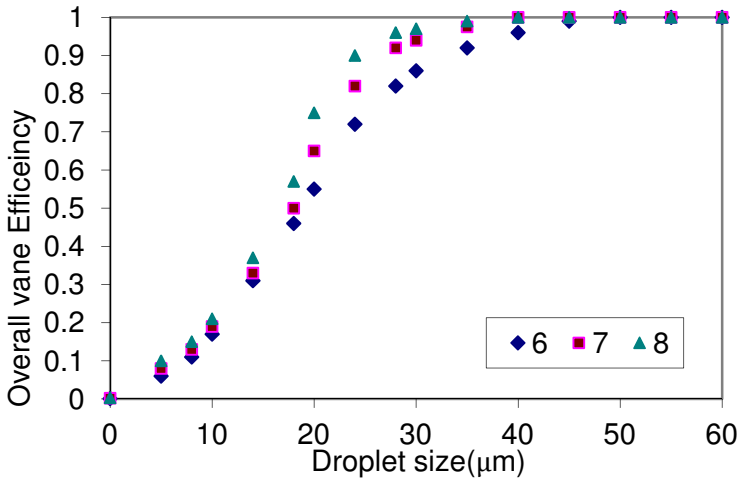

Figure (10) effect of varying the number of bends in vane pack on the overall vane efficiency

4.6. Vane Pack Bend Angle

From Figure (11), it is found that increasing the angle of vane pack bend will increase the overall vane efficiency as the gas and droplets have to bend through a greater angle. Therefore the number 80 degree is chosen as the optimum vane pack bend angle. The pressure drop for this case is around (10 pa) estimated from figure (4).

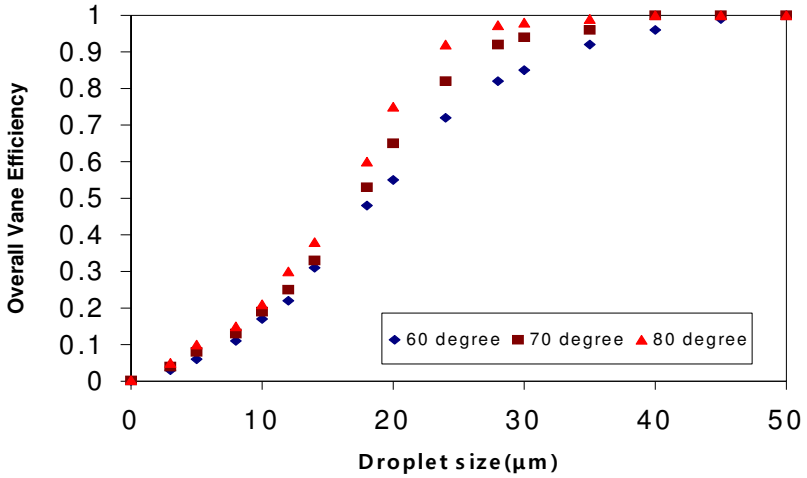

Figure (11) effect of varying the vane pack bend angle on the overall vane efficiency

4.7. Final Design

From the results of the investigations on the effects of changing various parameters on the separator efficiency, the following design as shown in table (4). An overall separation efficiency of 99.99% is achieved .The arrangement of the four vane packs in the separator is shown in Figure (12).

Table (4) final design parameters for the vertical phase separator

Overall design efficiency %	**99.99**
Vessel diameter (m)	2.7
Vessel height (m)	10.8
Pipe diameter (m)	0.813
Pipe bend radius(m)	1
Pipe bend angle	1.571
Vessel bend angle	1.571
Vessel bend spacing(m)	0.3
Momentum breaker bend radius(m)	1
Momentum breaker bend angle	3.142
Vane pack bend radius (m)	0.05
Vane pack bend angle	1.396
Vane pack bend spacing (m)	0.006
Number of packs	4
Pack depth(m)	1
Number of vanes per packs	200
Number of bends in packs	8

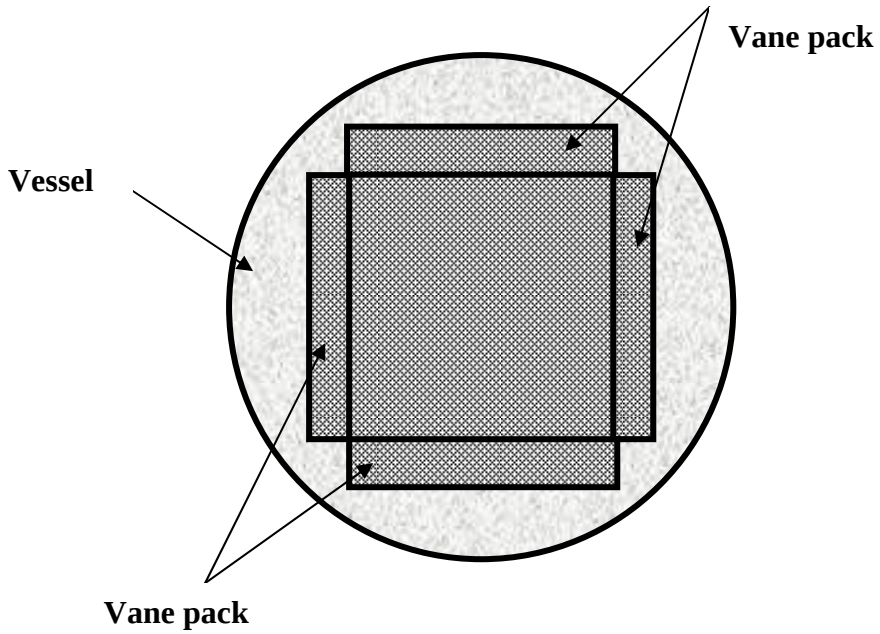

Figure (12) arrangement of vane pack from the top view of the vessel

5. Conclusion

An investigation has been undertaken to understand the effect of design parameter on vertical gas/liquid two-phase separator. From the data collected it can be concluded that:

- The efficiency increases with decrease in vane packs plate spacing.
- The efficiency of separator rises with an increasing in number of bends in the vane pack (up to 8).
- Increasing number of bends in pack increase the efficiency of separator.
- The efficiency of separator slightly increases with an increase in bend angle before separator, and the maximum efficiency of this case is 99.99%.

Abbreviations

η_O	Overall separation efficiency
η_F	Fractional separation efficiency
U_t	Settling velocity (m/s)
ρ	*L*iquid density (kg/m^3)
ρg	Gas density (kg/m^3)
D_v	Minimum vessel diameter, m
V_v	Gas volumetric flow rate, m^3/s
π	pi(3.14)
σ	Surface tension (N/m)
g	Gravity
U_g	Superficial velocity of gas (m/s)
L	Length of vessel, (m)
D	diameter of vessel, (m)
Mv	Gas mass flow rate , (t/h)
μv	Gas viscosity, (kg/ms)
P	Pressure, (bar)
T	Temperature, (C)

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کارتێکرنا دیزاینا پێڤانا پەستانا غازێ ل سەر شیانا ناڤبری ناڤ بەرا غاز/شل ل هێلا بوریێن غازێ

پوختە:

ئارمانجا سەرەکی یا ڤی کاری دە ست نیشانکرنا قەبارێ ناڤبرێ غازێ و شلێ بو ژێك جودا کرنا غازێ و شلێ ل سەر ماوەیەك دریژ ل هێلێن بوریێن غازێ. ئە ڤە کارەکێ فەرە بو کەرە رە نتیکرنا کارکرنا ئێمن بو پەستانێت ب کار اینایی لسەر هێلێن بوریا.

ناڤبرێ هاتی ئامادە کرنێ ب ژێکێشەرێ روی پێلەیی وەکی ناڤبرێ نەسەرەکی . دیزاین هاتییە ب دەستڤە اینان ب وردبینکرنێ دە ل پیڤەرێ غازێ ئەوا کارتێکرن هەیی لسەر ژێکجوداکرنا جوداکەری. دڤێ ڤێڤەکولینێدە دە, ستیراتیا بوریی یا ناڤخویی, ژێك دیربینا خواهربینا ئامانی, کوژیا خواهربینا پەرێ پانکێ, شلوڤەکەر, ژێك دیربینا پلیتێن پەرێ, ژمارا جەماندنان د کەرستێن هاتین ب کار اینان.

دڤێ ڤەکولینێدە ئەنجاما ٩٩,٩٩٪ ژ شیانا ناڤبرێ هاتیە ب دەستڤەئینان.

تأثير تصميم قياس ضغط الغاز على كفاءة الفاصل العمودي بين الغاز / السائل في خط أنابيب الغاز

الملخص:

الهدف الرئيسي من هذا العمل هو تحديد حجم المحور العمودي لفاصل الغاز و السائل لفصل الغاز والسائل على مسافة بعيدة في خطوط أنابيب النفط و الغاز. وهذا أمر ضروري لضمان التشغيل الآمن للضواغط المستخدمة على طول خطوط الأنابيب.

الفاصل المجهزة بساحب الرطوبة ذات السطح المتموج كفاصل ثانوي

تم تحقيق التصميم من خلال التدقيق في مقياس ضغط الغاز والتي لها تأثير على كفاءة فصل الفاصل. في هذه الدراسة، قطر الانبوب الداخلي، تباعد منحنى الوعاء، زاوية انحناء ريشة المروحة، المحلل, تباعد لوحات الريشة، وعدد الانحناءات في الحزمة. من خلال هذه الدراسة تم الوصول الى نتيجة ٩٩,٩٩٪ من كفاءة الفاصل..

SYNTHESIS AND SPECTROSCOPIC IDENTIFICATION OF A NEW SERIES OF BIOLOGICALLY ACTIVE 2-IMINOTHIAZOLIDINE-4-ONE DERIVATIVES

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(Accepted for publication: November 13, 2013)

Abstract

A series of 2-amino-5-(substituted phenyl) 1,3,4- thiadiazol have been synthesized through the reaction of thiosemicarbazide with substituted benzoic acids in the presence of phosphoroxy chloride readily undergo nucleophilic addition – elimination reaction with chloroacetyl chloride in benzene as a solvent to afford 2-chloro acetamido compounds. The prepared compounds were subjected cyclization reaction and results in the formation of a series of 2- imino-3-(substituted phenyl) 1,3,4- thiadiazol-2yl-thiazolidinone -4 one .

The IR ,^{1}H and ^{13}C- NMR spectra of the prepared compounds were confirmed to their proposed structures . Finally antimicrobial activity of the newly obtained compounds were tested against *Klepsilla pneumonia* (gram -ve) and *Staphylococcusaurous* (gram + ve) and the results showed that most of the prepared compounds are sensitive against both types of test organisms in different activities .

Key words: *iminothiazolidin-4-one, antimicrobial, addition–elimination*

1. Introduction

A heterocyclic compound (Morrison and Boyd ,1992) is one that contains a ring made up of more than one kind of atom. Heterocyclies (Chavan and Pai, 2007) bearing nitrogen, sulfur, and thiazole moieties constitute the core structure of a number of biological interesting compounds. The chemistry of thiazolidin-4-one ring system is one of considerable interesting as it is a core structure in various synthetic pharmaceuticals that displaying a broad spectrum of biological activities .Also 2-iminothiazolidin-4-ones have been found to have antifungal activity.

Design (Saeed et al. 2007) of general, simple and efficient methods for rapid synthesis of thiazolidinone would be greatly valuable and could warrant further investigations in drug discovery. Among (Turget et. al,2007) these types of molecules , 4-thiazolidinones have been shown to have various important biological activities such as bacterial antifungal and antiviral. Quantitative structure – activity relationship (QSAR) studies have also been performed on the basis of the fact that the biological activity of a compound is a function of it is physicochemical properties(Sharma et.al.,2009).

4-Thiazolidinones (Jubi et.al.,2009) are derivatives of thiazolidine with carbonyl group at the (4) position and formed by the attack of sulfur nucleophile on imine carbon followed by intramolecular cyclisation with the elimination of water. The derivatives of 4-thiazolidinone nucleus has occupied a unique place in the field of medicinal chemistry .

The synthesis of 2- iminothiazolidine -4-one has been (Singh et.al,1981) reported by using thiourea and sodium salt of the labled monochloroacetic acid .

2-Imino thiazolidinone were synthesized (Banday and Rauf,2009) from fatty acid hydrazides, firstly acylthiosemicarbazide was obtained which on cyclization of the latter compound with chloroacetyl chloride in chloroform give the desired 2-iminothiazolidinone .the aim of the our work is synthesize and spectroscopic identification of a new series of biologically active 2-iminothiazolidinone compounds.

2- Experimental

Instruments:

1- Melting points were determined by using electro thermal melting point apparatus from Stuart Scientific uncorrected.
2- IR spectrum were taken by Bio-rad Merlin FTIR spectroscopy ,Mod FTS 3000 .
3- The Nuclear Magnetic Resonance (300 MHz ^{1}H-NMR and 75MHz^{13}C-NMR) spectra were recorded on a Brucker using TMS as internal standard at Al-Albayat University-Jordon.

2.1 Synthesis of 2-amino-5-(substituted phenyl) 1,3,4-thiadiazols (1 a- h). (Jumaa,2005)

A mixture 0.02 mole of substituted benzoic acids, 0.02mole thiosemicarbazide and 10 mL of phosphoroxy chloride was placed in a 100 mL round bottom flask and refluxed for 0.5 h , the resulting reaction mixture cooled and followed by adding 24 mL of cooled water slowly. The obtained solution was further refluxed (4h). After completion of the reaction , the product was separated and washed with sodium carbonate solution (2.5 %) and water 2- times , then subject to dryness at r.t and recrystallized from (DMSO + H_2O) .The yields and melting points were summarized in Table (1) .

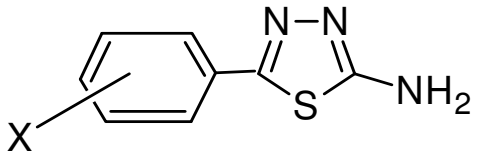
2.2 Synthesis of 2-chloro acetamido-5-(substituted phenyl) 1,3,4- thiadiazols (2 a-h).(Liu et. al,2000).

In a (100 mL) round bottom flask equipped with a dropping funnel and condenser,(0.01 mole) of 2-amino-5-(substituted phenyl) 1,3,4-thiadiazol was dissolved in benzene (30 mL) .The solution was cooled to (0-5C˚). After cooling, 0.01 mole of chloroacetyl chloride was added slowly to the mixture with vigorous stirring . After completion the addition , the reaction mixture was refluxed for 3h , and benzene removed under vaccuo . The residue was washed with 5% $NaHCO_3$,and subsequently with water, then dried and recrystallized from DMSO and water .The percentage of yields and melting points of the synthesized amides are shown in Table (2).

2.3 Synthesis of 2-imino-3-(substituted phenyl) 1,3,4-thiadiazol-2yl- thiazolidine-4ones (3 a-h)):(Liu et. al,2000).

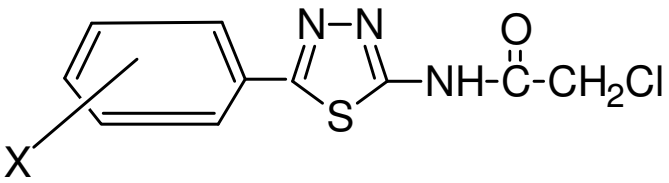
In a (50 mL) round bottom flask a mixture of 2- chloroacetatemido 5- (substituted phenyl) 1,3,4-thiadiazol (0.01 mole) and KSCN (0.01 mole) in acetone (33mL) was refluxed for 3 h. The excess of acetone removed under vacuum .The solid product was recrystallized from toluene. The percentage of yields and melting points of synthesized products are shown in Table (3)

Table (1): some physiochemical properties of prepared amines (2-amino-5-(substituted phenyl) 1,3,4-thiadiazols (1 a-h):



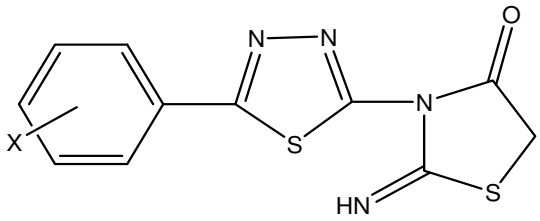
Compound	X	M .F	Yield %	M.P ˚C
1 a	4-CH_3	$C_9H_9N_3S$	62	214-216
1 b	4-OCH_3	$C_9H_9N_3OS$	85	228-230
1 c	3-Cl	$C_8H_6ClN_3S$	85	212-214
1 d	4-NO_2	$C_8H_6N_4SO_2$	83	226-230
1 e	3-NO_2	$C_8H_6N_4O_2S$	82	220-222
1 f	4-Br	$C_8H_6BrN_3S$	82	231-233
1 g	3-Br	$C_8H_6BrN_3S$	82	220-222
1 h	H	$C_8H_7N_3S$	83	174-176

Table (2): Some physiochemical properties of prepared amides (2-chloroacetamido-5-(substituted phenyl) 1,3,4-thiadiazols (2 a-h):



Compound	X	M.F of amides	Yield %	M.P °C
2 a	4-CH_3	$C_{11}H_{10}ClOS$	79	194-196
2 b	4-OCH_3	$C_{11}H_{10}ClN_3O_2S$	84	253-255
2 c	3-Cl	$C_{10}H_7Cl_2N_3OS$	83	174-176
2 d	4-NO_2	$C_{10}H_7ClN_3O_3S$	81	142-144
2 e	3-NO_2	$C_{10}H_7ClN_4O_3S$	81	190-192
2 f	4-Br	$C_{10}H_7BrClN_3OS$	83	190-192
2 g	3-Br	$C_{10}H_7BrClN_3OS$	83	194-196
2 h	H	$C_{10}H_8ClN_3OS$	83	228-230

Table (3): some physiochemical properties of prepared (2-imino-3-(substituted phenyl)1,3,4-thiadiazol-2yl thiazoilidin-4-ones(3 a-h) :

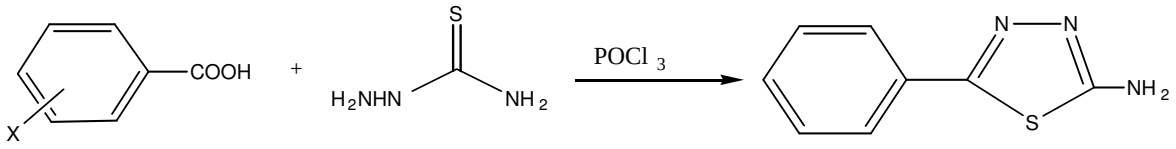


Compound	X	M.F	Yield %	M.P °C
3 a	4-CH_3	$C_{10}H_{10}N_4OS_2$	63	192-194
3 b	4-OCH_3	$C_{12}H_{10}N_4O_2S_2$	63	185-187
3 c	3-Cl	$C_{11}H_7ClN_4OS_2$	50	210-212
3 d	4-NO_2	$C_{11}H_7N_5O_3S_2$	44	190-192
3 e	3-NO_2	$C_{11}H_7N_5O_3S_2$	43	164-166
3 f	4-Br	$C_{11}H_7BrOS_2$	37	230-232
3 g	3-Br	$C_{11}H_7BrN_4OS_2$	35	98-100
3 h	H	$C_{11}H_8N_4OS_2$	35	200-202

Results and discussion

3.1 Synthesis of 2-amino -5-(substituted phenyl) 1, 3,4-thiadiazol (1 a-h) (Jumaa,2005) .

The reaction of substituted benzoic acids with thiosemicarbazide in the presence of phosphoroxy chloride gives 2- amino-5-(substituted phenyl) 1,3,4-thiadiazole. The products were identified by IR,[1] H-NMR, and [13]C-NMR .

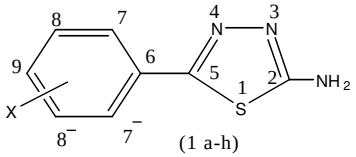


(1 a-h)

IR spectrum of 2-amino -5- (p-methyl phenyl) 1,3,4- thiadiazol (1 a) , Fig (1) (Table 4), observed several characteristic absorption bands at (3281 and 3081) cm^{-1} due to NH_2 group and at 2960 cm^{-1} due to C-H str. of the methyl group (Madkoub , 2004) at para position of the benzene ring and the disappearance of carbonyl group band for substituted benzoic acids is a good evidence for the elucidation of expected structure . The [1]H-NMR data(Table5),showed a signal at 2.3 ppm for methyl and amino groups at 7.4ppm . In addition , the aromatic protons were resonated in the aromatic region between 7.2-7.7 ppm(Chavan and Pai, 2007) .

The [13]C-NMR spectrum of compounds (1a) (Table 6),revealed the expected signal at 21.3 ppm (Moghaddam and Hojabri,2007) due to methyl carbon atom , and also two signals at (169 ,156) due to C_2 and C_5 of the thiadiazol ring , respectively .

Table (4): Assignments of characteristic frequencies (cm^{-1}) of IR spectra for the prepared amines (1a-h).



(1 a-h)

Compound	N-H Str.	C-H Aromatic Str.	C-H Aliphatic Str.	C=N str.	C=C Str.	NO_2 Str.
1 a	3281 , 3081	3040	2960	1637	1515	-------
1 b	3254 , 3101	3030	2950	1609	1513	-------
1 c	3400 , 3150	-------	--------	1622	1525	-------
1 d	3427 , 3230	3093	---------	1639	1595	1352,1525
1 e	3407 , 3280, 3159	3100	---------	1635	1527	1345,1535
1 f	3461 , 3290	3100	---------	1681	1599	--------
1g	3380 , 3180	-------	---------	1620	1523	------
1 h	3275 , 3100	3060	---------	1634	1514	-------

Table (5): [1] H –NMR data of prepared substituted amines (1a,b)

Compound	δ NH ppm	δ CH_3 ppm	δ OCH_3 ppm	δ Aromatic protons ppm
1 a	7.4(s,2H)	2.3(s,3H)		7.7 d (2H, 7,7¯), 7.2 d (2H, 8,8 ¯)
1 b	7.3(s,2H)		3.79(s,3H)	7.0 d (2H, 7,7¯) 7.7 d (2H, 8,8¯)

Table (6): ^{13}C -NMR data of compound (1a,b) :

Compound	C_2	C_5	C_6	$C_{7,7}$ -	$C_{8,8}$ -	C_9	CH_3	OCH_3
1 a	169	156	130.3	129.5	129.7	21.3	21.3	
1 b	168	161	156.7	130	115	160.7		56

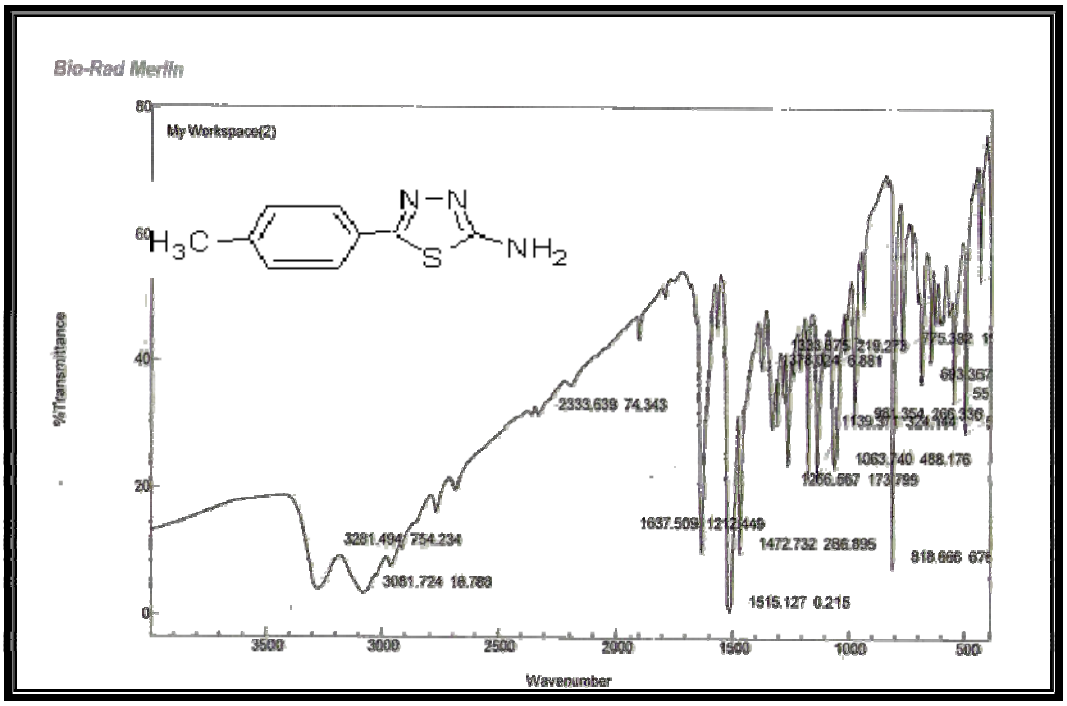
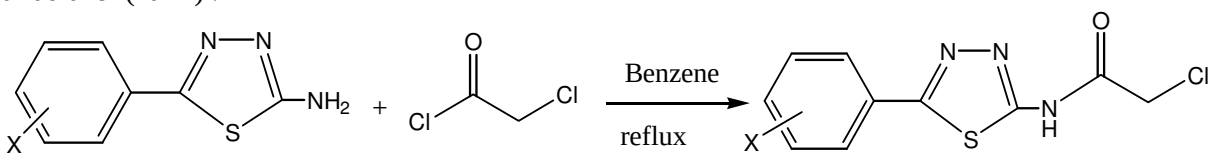


Fig (1): IR spectrum of compound (1a)

3.2 Synthesis of 2-chloro acetamido-5-(substituted phenyl)1,3,4- thiadiazols (2a-h) (Liu et.al.2000)

The most frequently used method for the preparation of amides is the reaction of amines and acylchlorides. In similar way , we focused on the synthesis of a series of 2-chloroacetamido 1,3,4-thiadiazol (2a-h) .



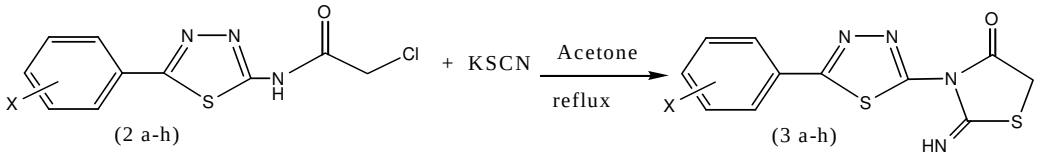
(1 a-h) (2 a-h)

2-Chloroacetamido-5-(p-methyl phenyl)-1,3,4-thiadiazol (2 a) was prepared from the reaction of 2-amino -5-(p-methyl phenyl) -1,3,4- thiadiazol and chloroacetyl chloride under reflux condition in benzene .

The infrared spectrum of 2-chloroacetamodo -5-(p-methyl phenyl) -1,3,4- thiadiazol (2 a) is shown in Fig. (2) and the most important feature of this spectrum is carbonyl stretch of the 2-chloroacetamido moiety at (1708) cm^{-1}(Chavan and Pai,2007) , Yadav(2005).The ^1H- NMR spectrum of (2a)(Fig 3) supported the expected structure by showing two singlet signals at (13) ppm(Chavan and Pai,2007) , due to N-H and at (2.3) ppm(Madkoub,2004) ,(Moghaddam and Hojabri,2007) (for methyl group , on the other band , the ^{13}C-NMR spectrum confirmed the ^1H-NMR finding by presenting two signals at (44) and (42.8) due to the 2-chloacetamido moiety.

3.3 Synthesis of 2-imino-3-(substituted phenyl)-1,3,4-thiadiazol-2-yl thiazolidin-4-ones (3 a-h) Jumaa,2005).

Since the nucleophilic substitution reaction of alkyl halides with potassium thiocyanate was successful especially in polar aprotic solvent , we decided to followed the same condition for carrying out reaction between (2 a) with potassium thiocyanate in acetone under reflux condition . In this reaction the in situ generated substitution product readily undergoes cyclization reaction to yield 2-imino -3-(p-methyl phenyl) -1,3,4 –thiadiazol-2-yl thiazilidin-4-ones (3 a) .



(2 a-h) (3 a-h)

The general feature of the IR spectrum of for compound (3a) exhibits a strong band at 1726 cm^{-1} (Table7) which belong to carbonyl groups of the 2-imino thiazolidin -4-ones structure(Sharma et al.2009;Abhinit et.al.2009) ,that is considered as evidence for the formation of the desired product , and strong band at 3253 cm^{-1} corresponding to NH str.(Abhinit et.al.2009 ; Makdoub 2004).

The Fig. (4) shows the ^{1}H-NMR data for some 2-iminothiazolidin-4-ones (3b) .The protons of CH_3 group are observed as a singlet signal at 3.85ppm (Jumaa 2005) , In addition the proton of (NH) group in thiazolidinone ring appears at (7.2) ppm (Yadav et.al.2005 ; Moghaddam and Hojabri,2007) , while the protons of aromatic rings appear at (7.06-7.92) ppm,(Makdoub 2004) and cyclic CH_2 appears as singlet signal at (4.27) ppm (Singh et.al.1981) . It seem from ^{13}C-NMR of some 2-iminothiazolidinones(3b), Fig. (5) , that the carbonyl of thiazolidinone compound (3b) appear at (174.5) ppm (Moghaddam and Hojabri,2007) which is the strong evidence for the forming of products. In addition to signal for(C=NH) carbon group appears (169.7) ppm(Singh et.al.1981;Aziz et.al.2009) and aromatic carbons appear at (114-161.8) (Aziz et.al.2009: Moghaddam and Hojabri,2007) .

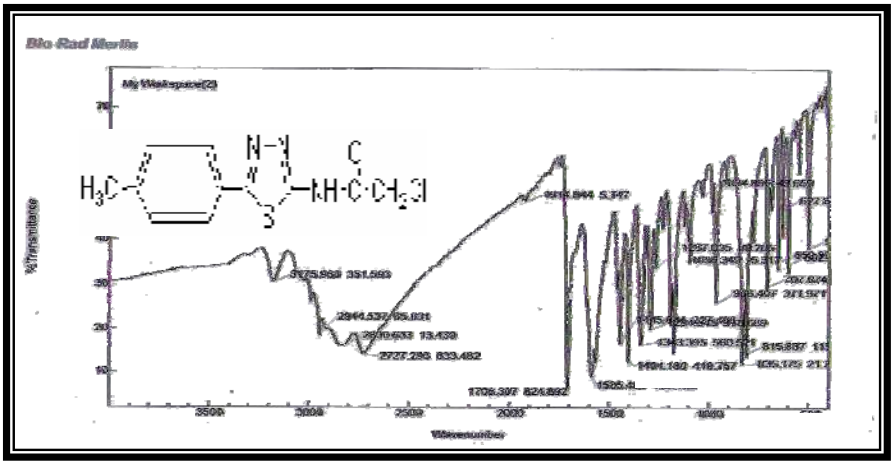
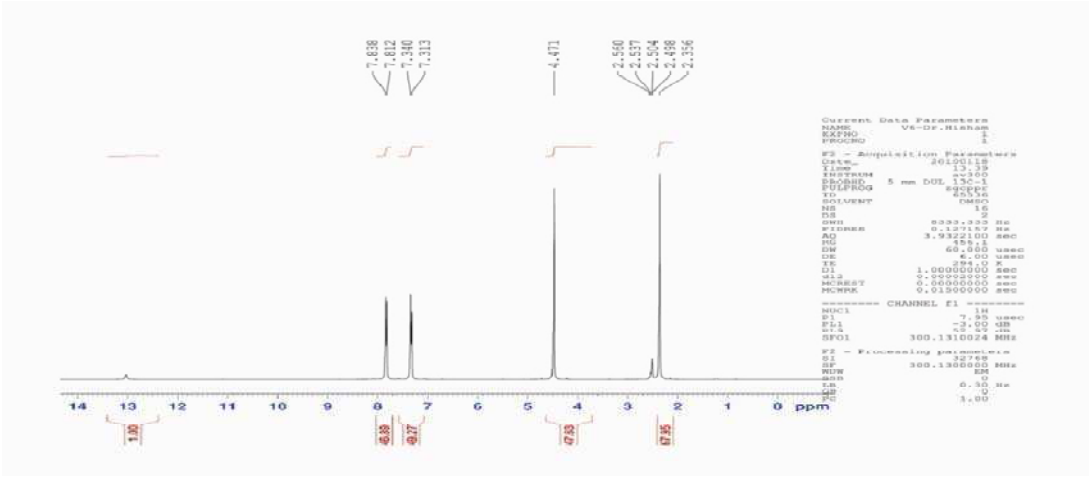
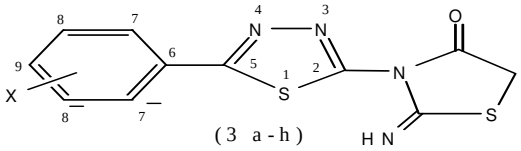


Fig (2): IR spectrum of compound (2 a)



Fig(3): ^{1}H-NMR spectrum of compound(2a)

Table (7): Assignments of characteristic frequencies (cm $^{-1}$) of IR spectra of the new 2-iminothiazolidin-4-ones (3 a-h).



(3 a - h)

Compound	N-H str.	C-H str. Aliphatic	C=O str.	C=N str.	C=C ring str.
3 a	3252	2924	1726	1632	1450
3 b	3437	2834	1736	1598	1580
3 c	3212	2987	1679	1501	1480
3 d	3200	2800	1679	1550	1590
3 e	3281	2987	1731	1614	1690
3 f	3200	-------	1700	1500	1550
3 g	3211	2981	1720	1570	1540
3 h	3440	2920	1715	1580	1540

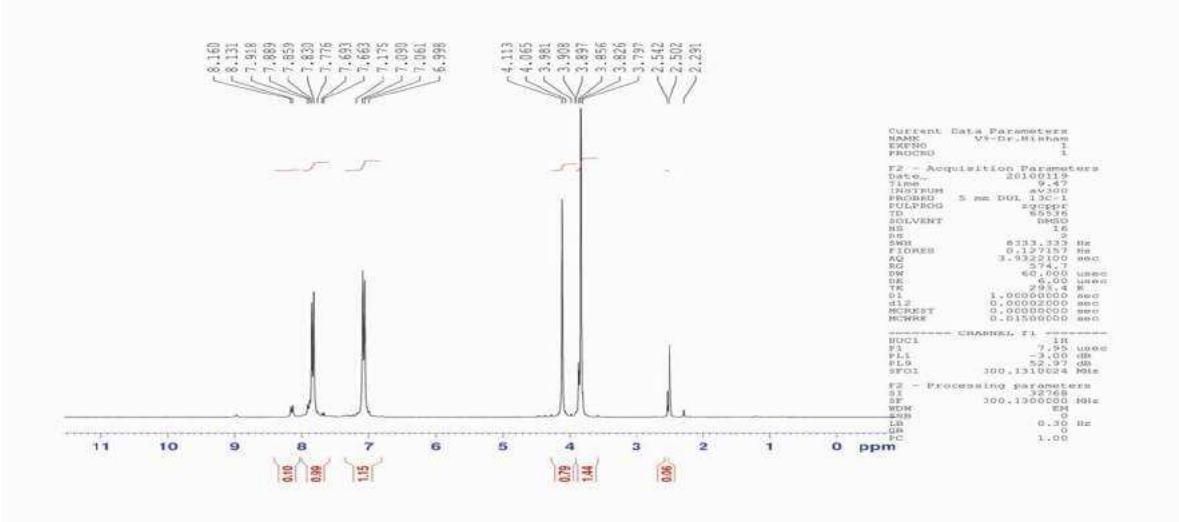


Fig (4): ^{1}H-NMR spectrum of compound(3 b)

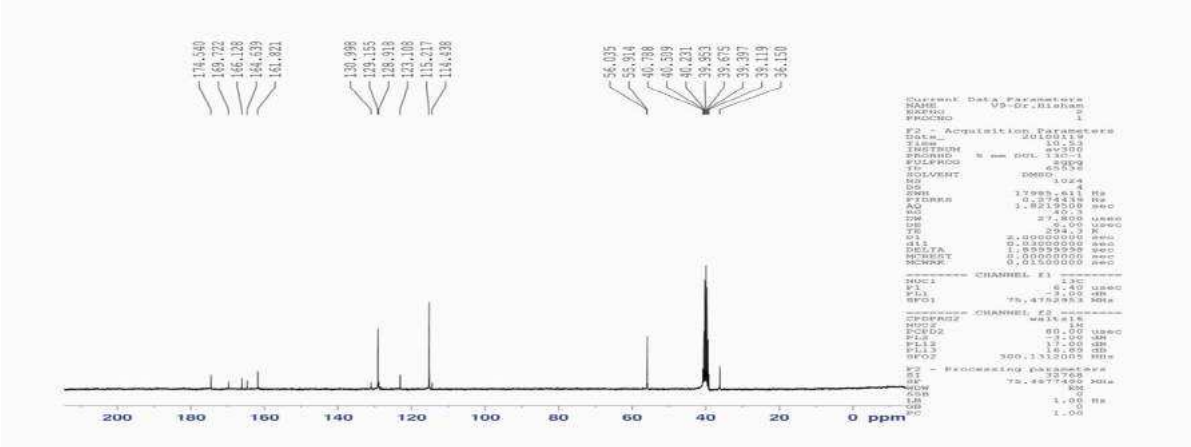


Fig (5) : C^{13} – NMR spectrum of compound (3b)

3.4. Anti-bacterial activities of the prepared compounds:

Some of the prepared compounds were screened for their antibacterial activity against two types of bacteria **Staphylococcus aurous** (gram positive) and **Kllepsilla pneumonia** (gram negative) using the cape late agar diffusion method . The prepared KBr discs of compounds(1:3) (the mixture was pressed under pressure to form discs) were placed on the surface of the cultured media and incubated for 24 hours at 37□C .Culturing the bacteria on nutrient agar, reading the zone inhibition: the larger zone of inhibition represents by more +ve but un effected by national committed for clinical laboratory.

During this study , it was found that the prepared compounds has anti-bacterial activity and their results were mentioned in (+) assignment (Table 8) .

Table(8): Anti-bacterial activities of some prepared compounds:-

Compounds	Microorganism	
	Kllebsilla pneumonia *–ve*	***Staphylococcus aurous*** *+ve*
1 a	+++	+++
1 c	+++	+++
1 d	++++	+++
1 e	++++	+++
2 a	++++	++++
2 f	+++	+++
3 a	+++	+++
3 b	+++	+++

Inhabitation zone (+) 7-10 mm , (++) 11-15 mm , (+++) 16-21mm, (++++) 22-28mm (Yadav et.al.2005)

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الخلاصة

يتضمن هذا البحث تحضير سلسلة من ٢-أمينو ١،٣،٤ ثايودايزول من خلال تفاعل ثايوسيمي كارباز ايد مع حامض البنزويك المعوض بوجود حامض فوسفوروكسي كلورايد والتي تعاني تفاعلات الاضافة والحذف مع كلورواسيتايل كلورايد في البنزين للحصول على 2- chloroacetamido 1,3,4- thiadiazol وبنتائج جيدة .

وفي مجال اخر في هذا البحث تم تحويل المركبات المحضرة بسهولة الى نظام حلقي لتعطي سلسلة جديدة من مركبات ٢- امينوثايزوليدين -٤- أون .

أخذت التحليل الطيفي (NMR, IR) للمركبات المحضرة لتاكد صحةالتراكيب المقترحة . و أخيرآ أختبرت الفعالية البيولوجية ضد نوعين مختلفين من البكتريا *Klebsiella pneumonia(gram –ve), Staphylococcusaurou*(gram +ve)و اظهرت النتائج بان معظم المركبات المحضرة لها حساسية ضد هذين النوعين من البكتريا و بفعاليات مختلفة .

پوختە

لەم توێژینەوە زانستی یە دا , زنجیرەیەك لە ٢- ئەمینۆ ٤,٣,١- سایەدایەزۆلّی ئامادە کراو لە کارلیّك کردنی سایۆسیمی کارباز اید لەگەلّ بەرخراوی ترشی بەنزویك بە بوونی فوسفورۆکسی کلۆراید لە جۆری کارلیّکی خستنە سەری و لیّکردنەوە بە ئاسانی لە گەلّ کلۆرۆئەسیتایل کلۆراید لە بەنزین بۆ بەدەست کەوتنی ٢-کلۆرۆئەسیتەمیدۆ کلۆراید بە بەرهەمێکی زۆرباش .

دواتر ئەمایدە ئامادەکراوەکان خرانە ناو کارلیّکی بەئەلقەبوون بۆ بەدەست هیّنانی زنجیرەیەکی نویّ لە ٢- ئیمینۆ سایۆزۆلیدین -٤-ئۆن .

شەبەنگی IR, ^{1}H-NMR, ^{13}C-NMR وەرگیرا بۆ ئاویّتە ئامادە کراوەکان وە راستی بەرهەمە ئامادەکراوەکانی دەرخست . لە کۆتایدا چالاکی دژە بەکتریا بۆ ئاویّتە بە دەست کەوتووەکان تاقی کرانەوە لە دژی *Klepsilla pneumonia*(-ve)و *staphylococcus aurous* (+ve)لە ئەنجامدا پیشانی دا کە زۆربەی ئاویّتە ئامادە کراوەکان هەستیارن بەرامبەر هەردوو جۆری بەکتریا یەکە بەلاّم بە چالاکی جیاواز.

DIAGNOSIS OF MINERALS CONTROLLED THE CARBONATE SOLUBILITY IN SOME CALCAREOUS SOILS FROM NORTH IRAQ

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Abstract

Four locations were chosen from North Iraq having different soil orders namely: (Zawaita-Mollisols), (Batail-Vertisols), (Ninevah forest-Inceptisols), (Tel-Afer-Aridisols) in order to study the role of solubility diagram and x-ray diffraction analysis in determining the type of mineral which effect the carbonate solubility.

Results showed that the calcite mineral effected the carbonate solubility depending on solubility diagram indicating by the points that falls under calcite mineral line represented the state under saturation with respect to this mineral. Also the results of x-ray diffraction stated the presence of calcite at d-spacing (0.304nm) and dolomite at (0.289nm), therefore the peak intensity of calcite (d-spacing) was sharpened and most height than the peak of dolomite, this conclusion was in agreement with the results of solubility diagram that were obtained above.

Keywords: *Minerals, Carbonate Solubility, Calcareous, X-Ray Diffraction.*

Introduction

Carbonate extent a major influence on the chemical properties of soils, for example adsorption and pH-related phenomena affect the availability of plant nutrients such as Zn and Fe, Mn (Bui *et al*., 1990). Calcium carbonate characterized by interaction with soil system, also their chemical and geochemical reactions interacted with other phases in soils which caused a large different in their properties especially the dissolution (Rezaei *et al*., 2004). This solubility is affected mainly by HCO_3^- and CO_3^{-2} concentration with effect on the chemistry of carbonate (Manahan 2005). Carbonate having different phases in soils named calcite, dolomite, aragonite, siderite, vaterite, magnesite depending on their solubility, but calcite and dolomite minerals formed a major carbonate phases in soils (Holtzclaw *et al*., 1986) and (Karl and Vodvic, 2000). The differentiation between these phases has important implication in soil genesis, chemistry and fertility (Holtzcalw *et al*., 1986). Langmuir, 1997 considered dolomite as one of the carbonate minerals common in sedimentary rocks, its solubility controlled by tow reactions:

1. Congruent reaction: happen in one step.

$$CaMg(CO_3)_2 \rightleftharpoons Ca^{+2} + Mg^{+2} + 2CO_3^{-2}$$

2. Incongruent reactions: happen in two steps.

$$CaMg(CO_3)_2 \rightleftharpoons CaCO_3 + Mg^{+2} + CO_3^{-2}$$
$$CaCO_3 \rightleftharpoons Ca^{+2} + CO_3^{-2}$$

Karbery *et al*., 2005 stated that the weathering and solubility of carbonate minerals is controlled by partial pressure of CO_2 according to Hennry's law which stated that the increase in carbonic acid is followed by increasing the rate of minerals weathering in soils. The chemical weathering of carbonate based on physo-chemical conditions of water and solid phase, with some parameters play an important role in the solubility of carbonate such as pH, PCO_2, ionic strength, Ca^{+2} ions, foreign ions (Abril *et al*., 2003).

The objective of this study was to differentiate the mineral which control the carbonate dissolution using solubility diagram and x-ray diffractometer (XRD).

Materials and Methods

This study was conducted on four locations from north Iraq having different soils orders namely: Zawaita-Molliosls, Batail-Vertisols, Ninevah forest-Inceptisols and Tel-Afer-Aridisols. These soils were classified at soil series (table 1) depending on (Soil Survey Staff, 1994) and (Al-Agaid, 1989).

Table (1): Soils classification at soil series

Location	Order	suborder	great group	series
Zawaita	Mollisols	Xerolls	Calcixerolls	653 CCE
Batail	Vertisols	Xererts	Chromoxererts	452 CCW

Ninevah forest	Inceptisols	Ochrepts	Xerochrepts	432 CCF
Tel-Afer	Aridisols	Orthids	Calciorthids	453 CCW

Therefore the soils samples were collected from horizons of each pedon and prepared for chemical analysis presented in (table 2) as described by Rowell , 1996.

Table (2) Some chemical characteristics of studied soils

depth (cm)	Horizon	pH	EC	Organic matter	Calcium carbonate	Active carbonate	CEC
Measurement unit			$dS.m^{-1}$		$gm.kg^{-1}$soil		$C.mol.kg^{-1}$
				Zawaita-653 CCE			
0-17	Ah	7.53	0.21	23.40	116.20	61.30	36.41
17-50	B_t	7.61	0.12	9.50	323.40	108.10	25.87
50-87	C_{1k}	7.70	0.11	8.10	369.60	87.21	19.65
				Batail-452 CCW			
0-18	Ap	7.48	0.17	15.80	123.20	102.60	32.75
18-44	B_{2t}	7.73	0.13	12.00	177.10	98,30	30.97
44-81	C_{1Ca}	7.52	0.19	11.00	231.00	112.40	25.86
				Ninevah forest-432 CCF			
0-22	Ah	7.40	0.17	19.40	111.00	27.80	18.54
22-48	(B)	7.64	0.13	12.00	127.60	38.90	14.32
48-80	BC	7.48	0.18	8.50	134.40	24.30	15.21
				Tel-Afer-453 CCW			
0-20	Ap	7.65	0.13	11.60	323.40	81.20	24.87
20-52	B_t	7.75	0.15	8.50	327.20	93.50	21.09
52-85	C_{1Ca}	7.81	0.31	8.20	351.60	108.60	20.98

Diagnosis of carbonate minerals:

I. Using Solubility diagram:

Dynamic equilibrium were made by preparing a soil suspension 1:20 (soil:water) at 298° Kelvin for 48hr. (Lindsay, 1979), after that the equilibrium extracts were collected to determine the electrical conductivity, pH, Ca^{+2} ion, as well as thermodynamic parameters were calculated as follows:

1- Ionic strength (I) using Griffin and Jurinak, 1973 equation.

$$I = 0.013 \times EC$$

2- Activity coefficient of calcium calculated by Davis equation.

$$-\log f_i = \frac{AZ_i^2\sqrt{I}}{1+\sqrt{I}} - 0.3\,I$$

3- Ionic activity

$$a_i = C_i \times f_i$$

4- Potential of calcium ion.

$$P_{Ca} = -\,\mathrm{Log}a_{Ca}$$

II. Using X-ray diffraction analysis:

Mineralogical analysis were made to differentiate carbonate minerals (calcite and dolomite) in soil samples firstly before dissolution and secondly after dissolution.

Firstly: Air dry soil samples represent the soil before carbonate dissolution.

Secondly: Soil samples represent the soil after dissolution, through an columns experiment (10 cm depth, 3 cm diameter) as follows. 40 gm of soil were added to each column and compacted to their bulk density, distilled-water was added everyday equal to porous volume of water. At the end of experiment (21 days) the soil samples were dried, grinded and sieved which formed the

soil after dissolution. The two samples were put in a special pellets of x-ray diffractometer and exposured to the x-ray at Θ angle between (26-34°).

Results and Discussion

To distinguish the minerals which control the carbonate solubility, thermodynamic parameters as shown in table (2) were used. Soils under study are non saline characterized by low levels of ionic strength ranged from 1.0-2.1mol.L^{-1}, the activity of coefficient ranged from 0.85 to 0.89, ion activity of calcium raised from 1.07 to 1.88mol.L^{-1}, the ionic potential of calcium are between (-logCa^{+2}) (-2.74) and (-2.97). all points of calcium ions potential were located under the line of calcite (Fig.1), reflecting an undersaturation of this minerals associated with calcite dissolution. The solubility and precipitation of carbonate minerals especially calcite is associated with downward movement of percolating water and upward by capillary as well as vertically and horizontally movement (Dunling and Anderson, 2000).

Table (3): Thermodynamic parameters using in solubility diagram

depth (cm)	Horizon	Ionic strength(10^{-3}) mol.L^{-1}	Activity coefficient of Ca^{++}	Activity of Ca^{++} (10^{-3}) Mol.L^{-1}	-logCa^{++}	pH
Zawaita-653 CCE						
0-17	Ah	1.5	0.86	1.31	- 2.88	7.6
17-50	B_t	1.7	0.85	1.88	- 2.74	7.5
50-87	C_{1k}	1.2	0.87	1.32	- 2.87	7.7
Batail-452 CCW						
0-18	Ap	1.8	0.85	1.28	- 2.89	7.7
18-44	B_{2t}	1.6	0.85	1.21	- 2.91	7.2
44-81	C_{1Ca}	2.1	0.84	1.28	- 2.89	7.7
Ninevah forest-432 CCF						
0-22	Ah	1.0	0.89	1.35	- 2.86	7.5
22-48	(B)	1.3	0.88	1.10	- 2.95	7.1
48-80	BC	1.1	0.86	1.34	- 2.87	7.3
Tel-Afer-453 CCW						
0-20	Ap	1.9	0.83	1.29	- 2.88	7.9
20-52	B_t	1.8	0.86	1.64	- 2.78	7.9
52-85	C_{1Ca}	1.7	0.85	1.07	- 2.97	7.4

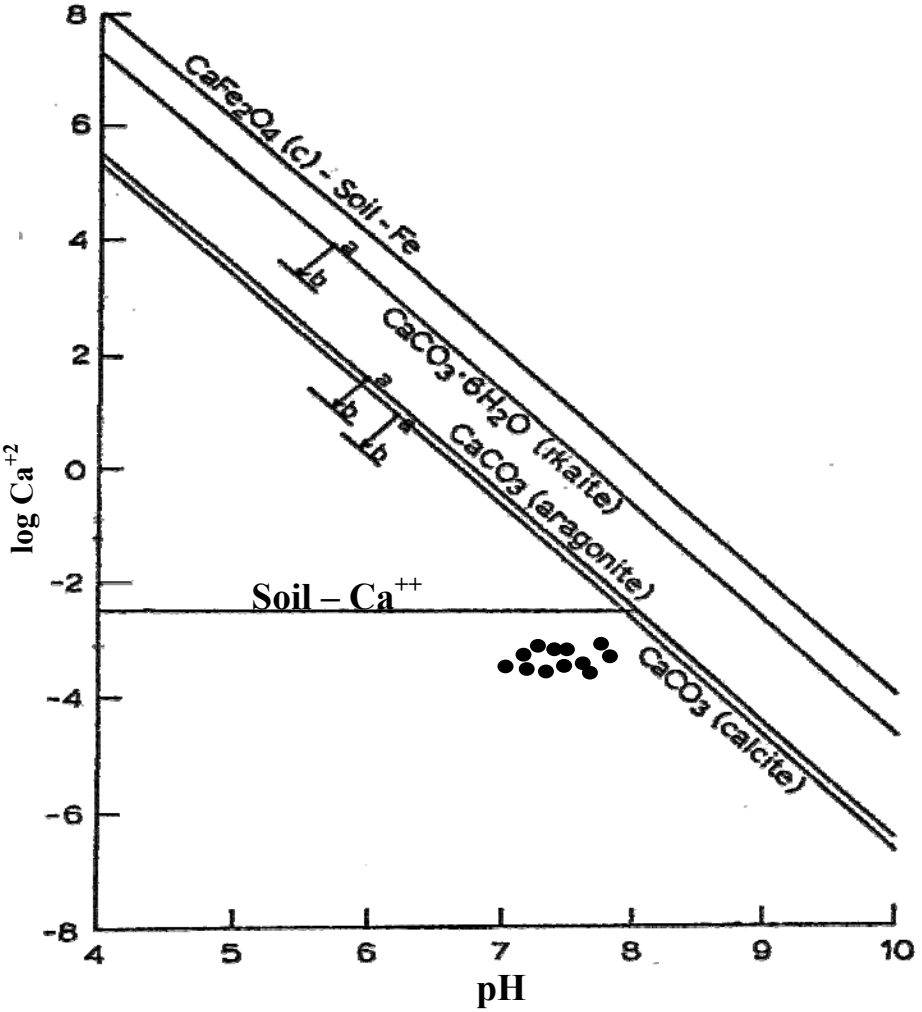

Fig. 1: Solubility diagram of carbonate minerals according to (Lindsay, 1979)

Clay minerals diffraction:

Carbonate minerals are numerous, some of them are common, others are unknown depending on their stability, some are unstable and transformed to other minerals, accordingly, they behave differently when analyzed with x-ray diffraction, calcite, the most common, show a d-spacing on 0.304nm, secondly Dolomite on 0.289nm. the main mineralongical feature of the studied soils are:

Zawaita region:

Figure (2) showed a sharp peaks at (0.304 nm) for calcite with heights (4.5, 8.8 cm) at subsequent horizons after dissolution, dolomite peaks dosppeared in the surface horizon before and after dissolution giving peaks of subsequent depths at (4.5, 5 cm) height. This indicated that dolomite was the dominant mineral at deeper horizons especially C_k horizon (Reeder, 1983).

Batail soil:

Figure (3) indicated that peaks of dolomite didn't appear in recognized horizons before and after dissolution, but calcite gives a clear peaks at (4, 4.8, 5.5 cm) heights after dissolution. The differences in peaks intensity after and before dissolution is because these soils are characterized by height content of swelling-type clays, which in dry seasons cause the soils to develop deep, wide cracks, associated with
a phenomena called pedoturbation. (Dunling and Anderson, 2000) stated that peaks intensity of calcite increase with decreasing dolomite presence in soil.

Ninevah forest soil:

As shown in (Fig. 4) the peaks of calcite appeared at (0.304 nm) before dissolution with lower intensity in the studied profiles reflected by peaks height of (2, 2.6 cm). after dissolution the Calcite appearance increased at the surface layer and 48-80 cm depth,

reaching a 5.0cm height. This increment is due to the removal of precipitated non-crystalline particles on the surface of calcite mineral causing an increase in intensity of crystalline mineral. In addition this increase resulted from precipitation of secondary carbonate on the surface of coarse particles as coatings or as individual districts due to pedogenic processes (Miller *et al*., 2000) and (Al-Kaysi, 2000).

Tel-Alfer soil:

The height of calcite peaks were (5.5, 5.5, 6 cm) at the surface, (20-52 cm) respectively before dissolution (Fig. 5). So the dissolution increased the heights to (8.5, 8.5, 9 cm). The results indicated that calcite was dominant correlated with clay content, therefore the expectation of calcite dominany is to take into consideration since carbonate in clay particles is pedogenic as shown by (Dunling and Anderson, 2000) that 72-100% of carbonate in clay separates has a pedogenic origin. The disappearance of dolomite indicated that it is inherited from parent material (Gile, 1995).

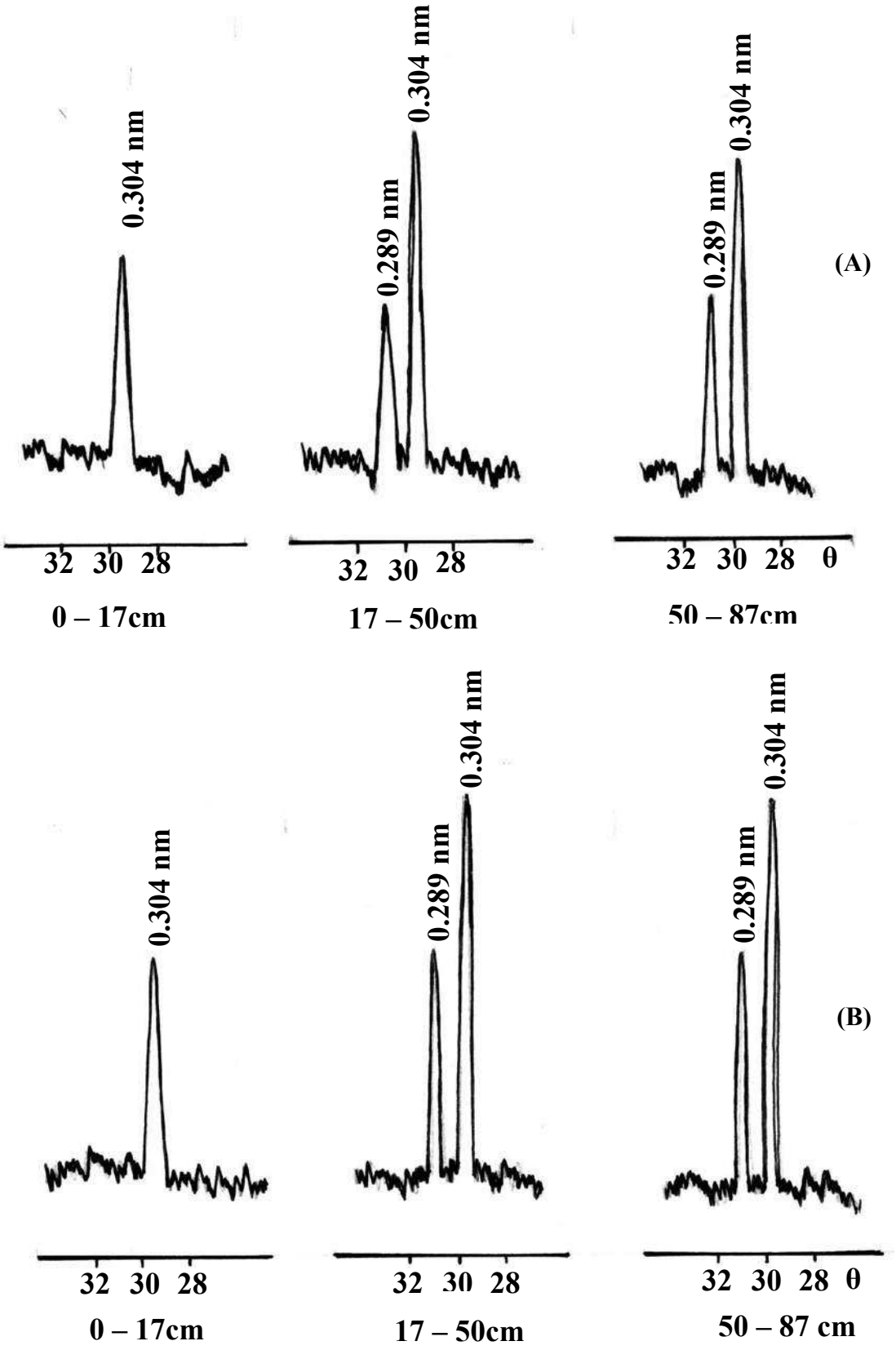

Fig. 2: x-ray diffraction of carbonate minerals – Zawaita soil

A- before dissolution.

B- after dissolution.

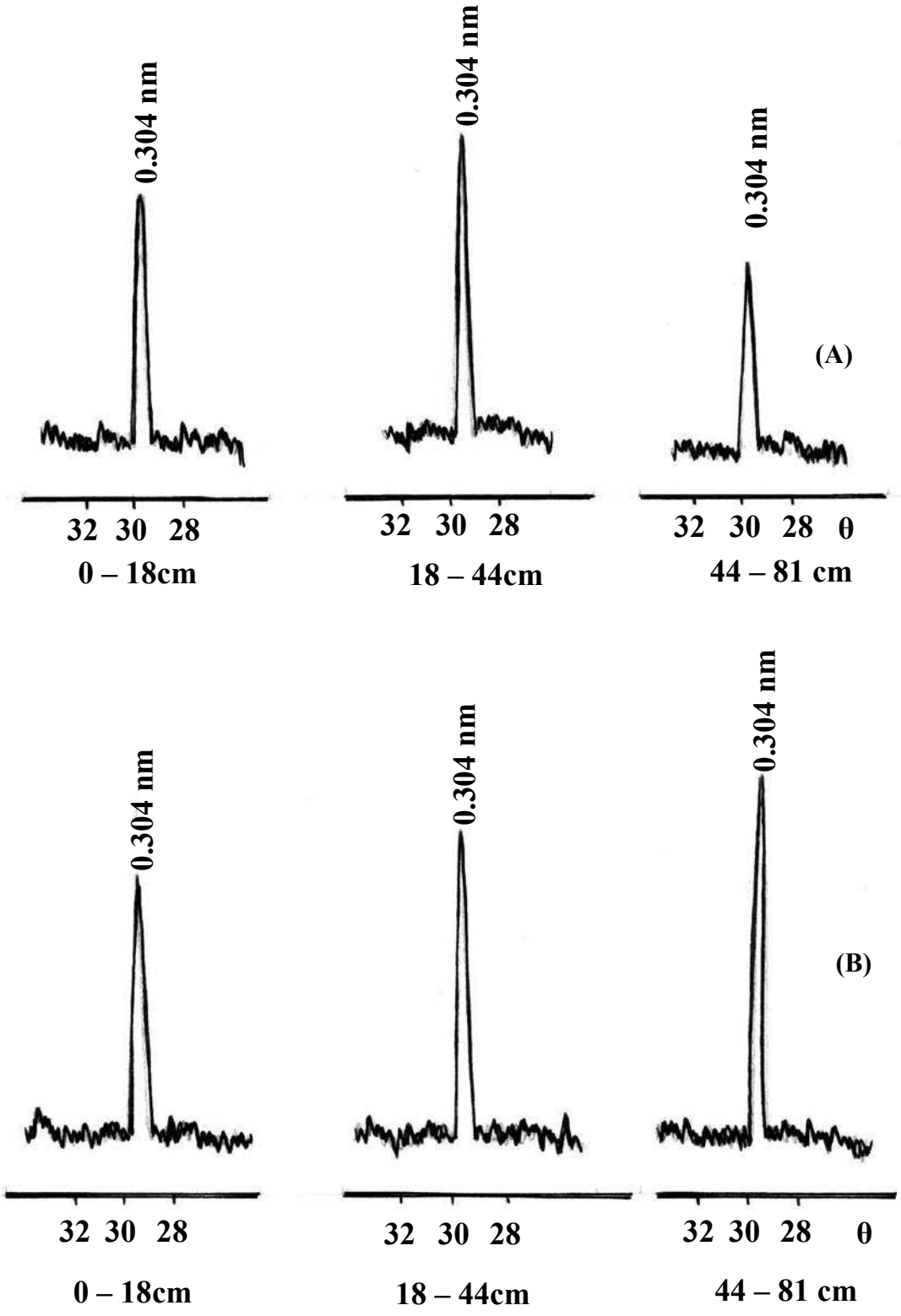

Fig. 3: x-ray diffraction of carbonate minerals – Batail soil

A- before dissolution.

B- after dissolution.

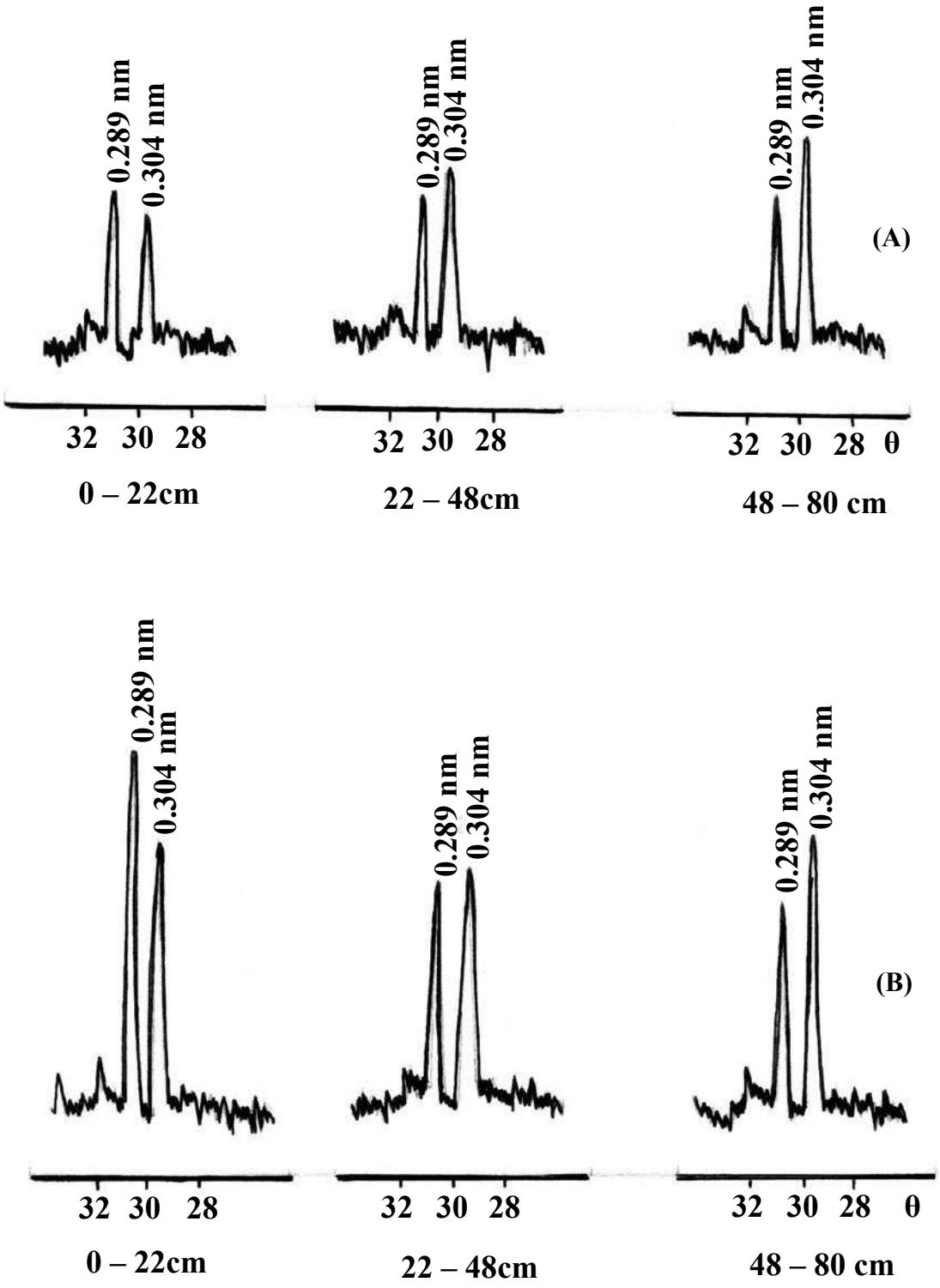

Fig. 4: x-ray diffraction of carbonate minerals – Ninevah forest soil

A- before dissolution.

B- after dissolution.

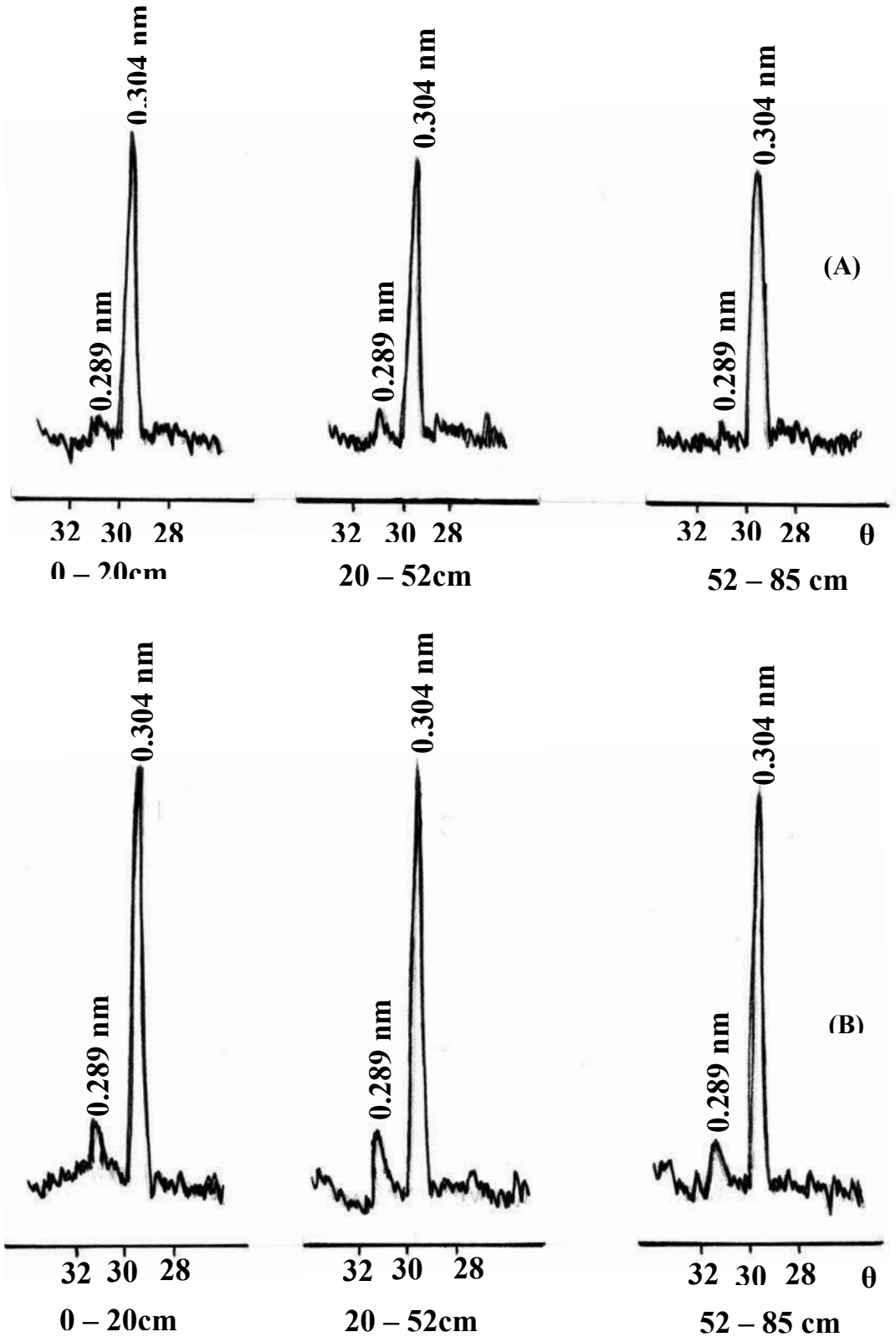

Fig. 5: x-ray diffraction of carbonate minerals – Tel-Afer soil

A- before dissolution.

B- after dissolution.

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تشخيص المعادن المحددة لذوبانية معادن الكاربونات في بعض الترب الكلسية في شمالي العراق

الملخص

اختيرت اربعة مواقع من شمالي العراق مصنفة بمستوى السلسلة تمثل ترب (زاويتا – Mollisols وباتيل Vertisols وغابة نينوى Inceptisols وتلعفر Aridisols ، لغرض دراسة دور مخططات الاذابة وحيود الاشعة السينية في تحديد نوع المعادن المتحكمة بذوبانية معادن الكاربونات.

اظهرت النتائج بان معدن الكلسايت هو المتحكم بعملية ذوبان الكاربونات . وان مخططات الاذابة بينت بان جميع مواقع الدراسة كانت تحت خط ذوبان معدن الكلسايت ، كما اشارت نتائج التحليل المعدني باستخدام XRD الى تشخيص معدن الكلسايت عند d– Spacing ٠,٣٠٤nm ومعدن الدولومايت عند ٠٢٨٩ nm واتصفت منحنيات الحيود لمعدن الكلسايت بكونها حادة وكانت اكثر طولاً من منحنيات الحيود لمعدن الدولومايت.

WATER QUALITY OF DOHUK DAM STREAM OUTFLOW

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Abstract

The Dohuk dam is located on the north of Dohuk city, it is consider as one of the main sources for water supplying to many places in the city for drink and domestic uses, dam water flowing through the narrow stream within the Dohuk valley finally reach to the Mosul dam. The study covered three locations from the Dohuk dam outflow stream, first from the bottom tunnel of the dam, second in Bourse bridge and third in Church bridge, the samples were taken from October 2010 until February 2011. The research aimed to water quality monitoring by determination some of physical, chemical and biological properties with respect to its potability for human uses.

The electrical conductivity (EC) and total dissolved solids (TDS) values of water reach to 1116 µs/cm and 899 mg/l, pH value was 9.1 in Bourse bridge location. Water of the Dohuk dam outflow stream was on the alkaline side. Total hardness recorded 603 mg/l CaCO3 in Church bridge location. The water of Dohuk dam outflow stream was classified as hard water as its hardness was higher than 300 mg/l (WHO, 2006).

The Dissolved oxygen (DO) values were decreasing through the Dohuk dam outflow stream to reach 6.0 mg/l in Church bridge location. The values of Biochemical Oxygen Demand (BOD) increasing through the dam outflow stream to reach 4.1mg/l in Church bridge location, the number of bacterial colonies also increasing in the stream flow which was reached to 432 cells/ml in Church bridge location.

Key words: Dohuk dam, water quality, monitoring water properties, domestic discharge.

Introduction

The freshwater for human consumption comes from rivers, lakes and underground. These sources account for only one percent of all water on the earth (Gebrekidan and Samuel, 2011). It is able to dissolve, adsorb or suspend many different compounds (WHO, 2007).Thus, in nature, water is not pure as it acquires contaminants from natural and anthropogenic sources (Momodu and Anyakora, 2010).

The presence of metals in water results from two independent factors. The first involving the weathering of soils and rocks with its products being transported by air and water, and the second involving a variety of anthropogenic activities that have created and a societal health risk in rivers that receive a substantial amount of waste (Roberto *et al.*, 2008).

Quality drinking water is essential for life, good drinking water quality is essential for the wellbeing of all people. Contaminants such as bacteria, viruses, heavy metals, nitrites and others found their way into water supplies as a result of inadequate treatment and disposal of solid and liquid waste (human and livestock), industrial discharges, and over-use of limited water resources (Singh and Mosley, 2003).

This paper focus on the water quality of Dohuk dam outflow stream by selected three locations.

Methods and Materials

The studied area include water discharged from Dohuk dam that moved with stream reach to the Dohuk valley and finally reach to Mosul dam, the study based on three locations from the Dohuk dam outflow stream, first from Dohuk water dam after flowed and second location of this water in Bourse bridge then third in Church bridge through October 2010 to February 2011. All water samples were kept in polyethylene bottles (Stankovic *et al.*, 2007).Water samples were tested the, electrical conductivity, total dissolved solids, pH, dissolved oxygen, BOD, total hardness and bacteria test. The tests were conducted according to the following methods (APHA, 1999)

Electrical Conductivity (EC)

Conductivity was estimated by electrical conductivity meter, "InoLab EC, TDS Level 1, HANNA instrument, WTW ", the prop was calibrated monthly by buffer solution, on the reading the conductivity values were converted to specific conductivity at 25C^{o} and the results were expressed by as µs/cm.

Total Dissolved Solid (TDS)

The amount of the total dissolved solids in water was estimated by TDS meter "InoLab EC, TDS Level 1, HANNA instrument, WTW ".

Hydrogen Ion Concentration (pH)

The pH was measured directly by using portable pH meter "InoLab pH Level 2, HANNA instrument, WTW", pH meter was calibrated with three buffer solutions of pH 4,7and 9.

Dissolved Oxygen (DO)

Determination of oxygen was carried out according to the Winkler's method (Azide modification) as describing by (APHA, 1999) the results were expressed in mg/l.

Biochemical Oxygen Demand (BODs)

The water sample saved in incubator during 5 day under 20C^{o} after that determine dissolved oxygen by Winkler's method (Azide modification) as describing by (APHA, 1999), the results were expressed in mg/l.

Total Hardness

Estimated of total hardness was made by titrating water samples against EDTA disodium salt with Eriochrome black T indicator at pH 10 (using ammonium buffer) the results were expressed in mg/l CaCO3 (APHA, 1999).

Bacteria Test

Estimated by Total Plate Count (TPC) (APHA, 1999).

Results and Discussion

The Electrical conductivity of water clarify from the Table 1, that the minimum value of 801µs/cm was recorded in Bourse bridge location and maximum value of 1116 µs/cm in Church bridge location. Fig.1 elucidate increasing mean of EC through the dam outflow stream to reach 937µ s/cm in Church bridge location this increasing occurred as a result to the domestic wastes discharge to this stream (Fatoki *et al.*, 2001), and the interactions between the acidic compounds which form from oxidation decomposition processes with basic compounds that found as suspended materials (Al-Saffawi *et al.*, 2009).

Table (1): Demonstrate the minimum, maximum and mean values of study parameters in study sites.

Parameters / Sites		EC µs/cm		TDS mg/l		pH		Total Hardness CaCO3 mg/l		DO mg/l		BOD mg/l		Bacterial colonies TPC Cells /ml	
Dohuk dam	min	803	min	620	min	7.4	min	340	min	7.7	min	1.0	min	53
	max	1004	max	781	max	8.0	max	471	max	9.2	max	1.4	max	92
	mean	871	mean	688	mean	7.7	mean	419	mean	8.4	mean	1.2	mean	76
Bourse bridge	min	801	min	625	min	8.3	min	322	min	6.1	min	2.5	min	138
	max	1126	max	857	max	9.1	max	587	max	9.0	max	3.2	max	197
	mean	907	mean	721	mean	8.5	mean	430	mean	7.3	mean	3.0	mean	169
Church bridge	min	816	min	679	min	8.2	min	408	min	6.0	min	3.3	min	246
	max	1116	max	899	max	8.7	max	603	max	7.8	max	4.1	max	432
	mean	937	mean	785	mean	8.5	mean	487	mean	6.7	mean	3.8	mean	408

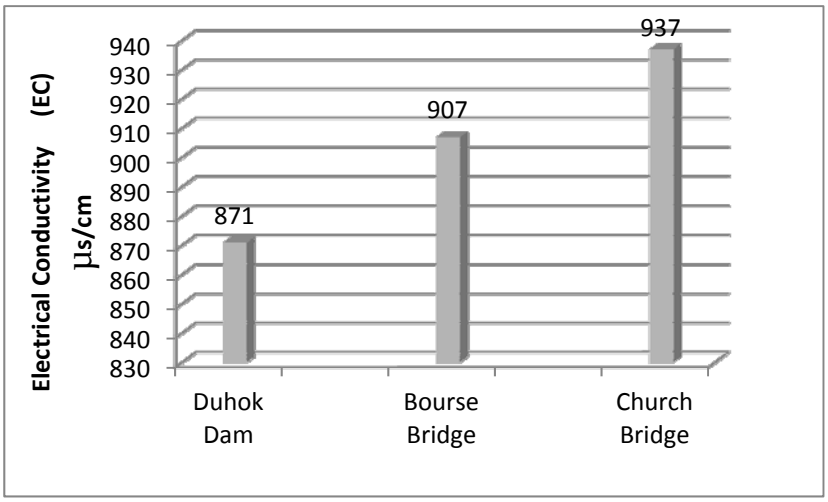

Figure (1): The mean value of (EC) in study locations.

The total dissolved solids of water represented in the Table 1, the minimum value of 620 mg/l was recorded in Dohuk dam location and maximum value of 899 mg/l was in Church bridge location, it is appear from Fig.2 that the increasing mean of TDS through the dam outflow stream to reach 785 mg/l was in Church bridge location caused by the effects of liquid wastes reach to it. All of the recorded values were above the minimum level of drinking water standard for drinking recommended by WHO and EPA (500 mg/l) (WHO, 1993; EPA, 2006).

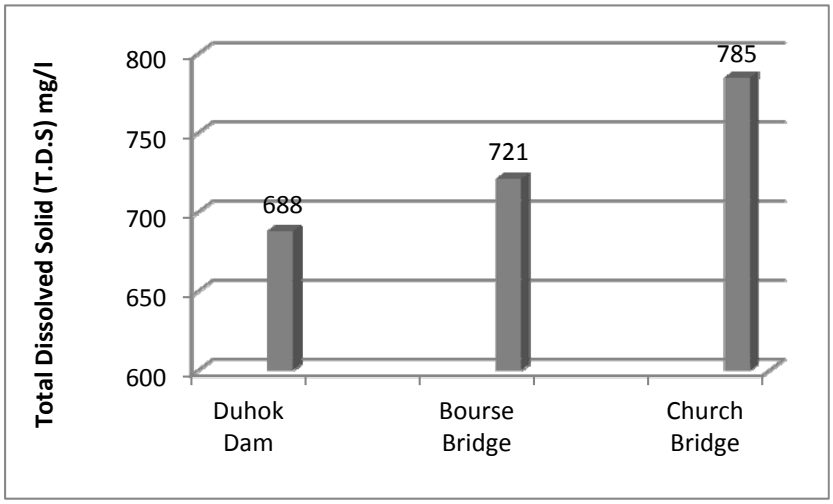

Figure (2): The mean value of (TDS) in study locations.

pH values of water represented in Table 1, the minimum value of 7.4 was recorded in Dohuk dam location, while maximum value of 9.1 was recorded in Bourse bridge location. Fig.3 illustrated mean of pH through Dohuk dam outflow stream to reach 8.5 in Bourse and Church bridge locations, the water of the dam outflow stream was on the alkaline side (7.4 - 9.1), this may due to the presence of carbonate and bicarbonate as dependent on the geology of area in additional to the nature of pollutants which reach to the stream such as detergents that has alkaline effect on the water (Adefemi *et al.*, 2007; Akoto and Adiyiah 2007).

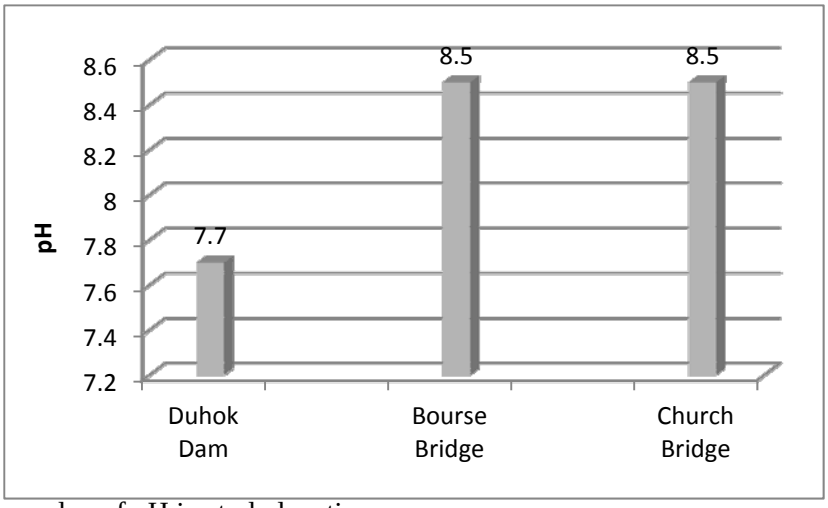

Figure (3): The mean value of pH in study locations.

The minimum value of total hardness was recorded in Bourse bridge location (322 mg/l CaCO3) and maximum value of (603 mg/l CaCO3) was recorded in Church bridge location. Fig.4 illustrated increasing mean of total hardness through the dam outflow stream that reach 487 mg/l CaCO3 was in Church bridge location**.** This increasing occurred as a result to the domestic and agricultural wastes discharge to this stream (Al-Barware and Al-Jahssany, 2009; Al-Saffawi *et al.*, 2009) and wastes from soil during the rain seasons, the variation of hardness is probably related to the geological formation of the area (Al-Negashabandi, 2002; Shihab and Abdul Baqi, 2010; Adefemi *et al.*, 2007), most values of water total hardness was lower than the hardness recommended by WHO of 500 mg/l CaCO3. The water of Dohuk dam outflow steam was classified as hard water as its hardness was higher than 300 mg/l. (W.H.O, 1993).

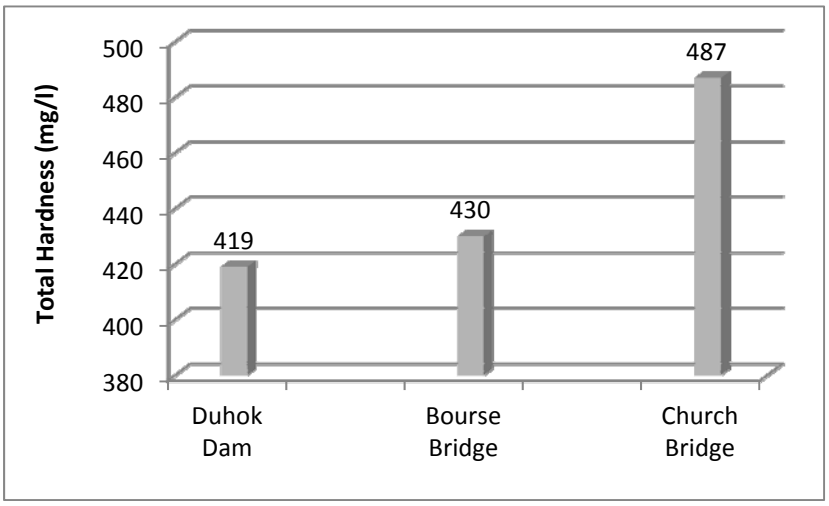

Figure (4): The mean value of Total hardness in study locations.

The Dissolved oxygen values of water represented in Table 1, the minimum value of DO was 6.0 mg/l recorded in Church bridge location and maximum value of 9.2 mg/l was in Dohuk dam location. Fig.5 illustrated decreasing mean of DO through the dam outflow stream to reach 6.7 mg/l in Church bridge location this decreasing occurred as a result to the oxidation and decomposition processes for organic materials by bacteria, The increase domestic and agricultural wastes into the environment helps the growth of bacteria by oxidation processes (Al-Jahssany, 2003; Al-Barware,2004).

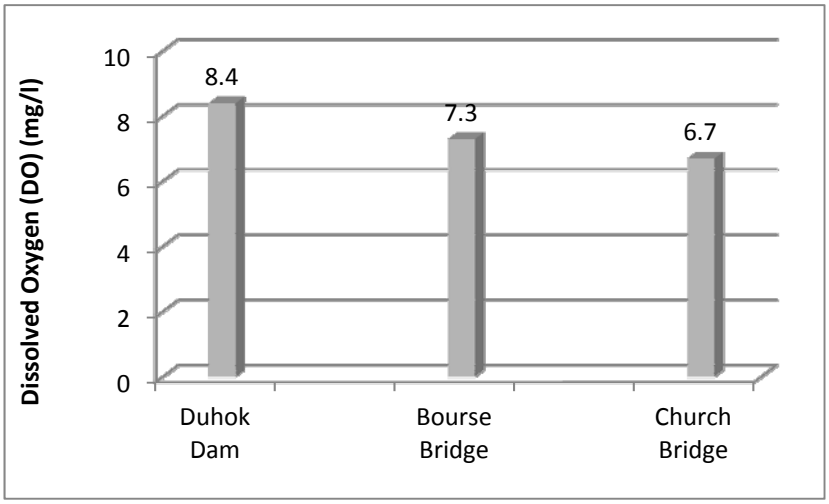

Figure (5): The mean value of (DO) in study locations.

Biochemical Oxygen Demand (BOD) values of water represented from the Table 1, the minimum value of 1.0 mg/l was recorded in Dohuk dam location and maximum value of 4.1 mg/l was in Church bridge location. Fig.6 illustrated increasing mean of BOD values through the dam outflow stream to reach 3.8 mg/l in Church bridge location. This increasing result from the domestic and agricultural wastes discharge to the dam outflow stream which contain organic matter, contain different types pollutants and detergents in additional to residual dead algae through the stream (Osibanjo *et al.*, 2011; Soylak *at el.*, 2002).

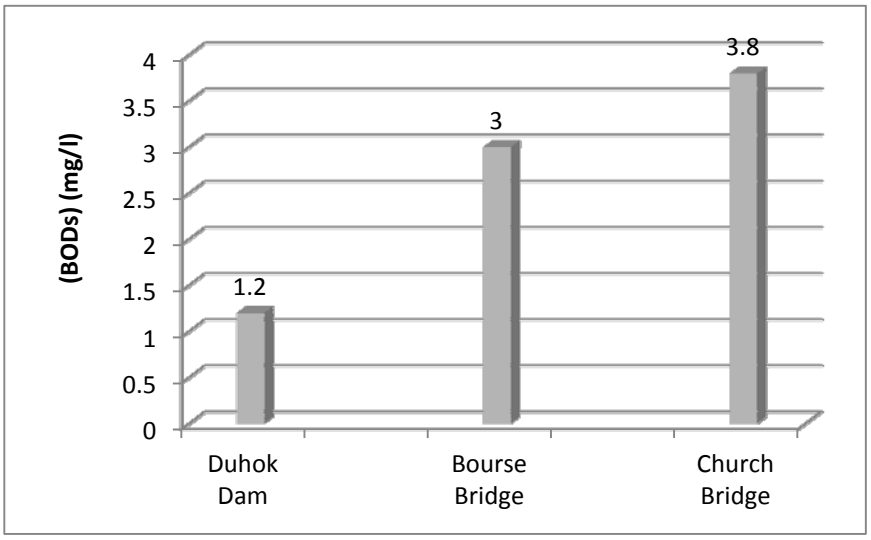

Figure (6): The mean value of (BOD_5) in study locations.

The number of bacterial colonies in water represented in Table 1, the minimum colonies was recorded 53 cells/ml in Dohuk dam location and maximum colonies was 432 cells/ml in Church bridge location. Fig.7 illustrated increasing mean of bacterial colonies through the dam outflow stream to reach 408 cells/ml in Church bridge location as results from the effluents organic materials which help to growth and reproduction of bacteria during to decomposition of deed organisms and this organic materials (Al-Tayyar *et al.*, 2008; Al-Saffawi *et al.*, 2009), also bacteria may be enter from water-legally, accidentally and through illegal dumping (Al-Shawani, 2001; Al-Jahssany, 2003).

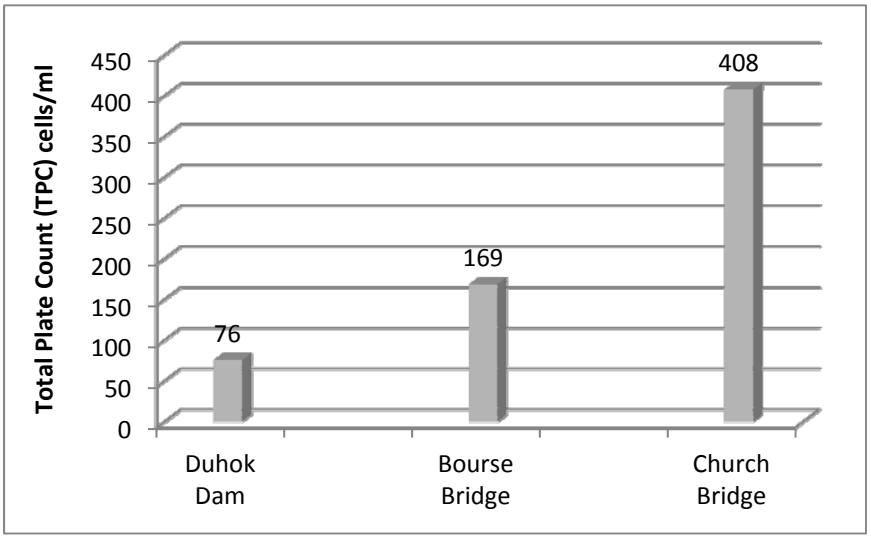

Figure (7): The mean value of bacterial colonies in study locations.

Conclusion

1-The water of Dohuk dam outflow stream recorded high value of (EC) as a result of domestic waste discharge.
2-The total dissolved solids values were above the minimum permissible level of water standard for drinking that recommended by WHO and EPA of 500 mg/l.
3-The water of the Dohuk dam outflow stream was in alkaline side.
4-The water of Dohuk dam outflow stream classified as hard water.
5-Bacterial colonies in the water increasing as results of domestic waste which help growth and reproduction of bacteria.

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لێکولینەك لسەر کوالیتیا ئاڤا ئاڤەرویێن سکرێ دهوك

پوختە:

سکرێ دهوك دکەڤیتە باکورێ پارێزگەها دهوك، کو ئێکە ژ ژێدەرێن سەرەکی بۆ دابینکرنا ئاڤێ بۆ ژمارەکا دەڤەرێن ناف شارێ دهوك بۆ مەرەما ڤەخارنێ و پێتڤیێن روژانە ، ژێدەرێن ئاڤا سکری ژ سەروکانیەکا کانییێت بچیك کوم دبیت هەتا دگەهیتە گەلیێ دهوکێ ناف باژیری هەتا دگەهیتە ناف سکرێ میسل، بۆ ڤێ لێکولینی سێ جھ هاتبونە دەست نیشانکرن ، جهێ ئێکێ ل ژێر نەفەقا سکری، یێ دووێ ل ژێر پرا بورسێ ،و یێ سێیێ ل ژێر پرا کەنیسێ، ئاڤ هاتە وەرگرتن ژ هەیڤا دهە ۲۰۱۰ هەتا هەیڤا دوو ۲۰۱۱ ئامانج ل ڤێ ڤەکولینێ چاڤدێری کرن و دەست نیشانکرنا خسلەتێن فیزیاوی و کیمیاوی و بایلوجی کەرەستێن ئەندامی یێن ئاڤێ بهێنە دەست نیشانکرن و ئاستێ پاقژیا ئاڤێ بۆ بکارئینانێ ژ لایێ هاولاتیا.

ئەنجامێن گەهاندنا کارەبایی و کەرەستێن رەق گەهشتنە ۱۱۱٦ مایکروسیمینز/سم ، ۸۹۹ ملگم/لتر ل دویف ئێك، ئەنجامێ ئوسا هایدروجینی 9.1 پلە تومارکرن ل جهێ پرا بورسێ ، و ئەنجاما دیارکرن کو بەرەف تفتیڤە، و ئنجامێن چراتی گشتی ٦۰۳ملگم/لتر پلە هاتنە تومارکرن ل دویف ئێك جهێ پرا کەنیسێ، ئاڤا ڤان ئاڤەرویێت هاتینە بەحسکرن ریژەکا یا عوسرێ تێدابوو کو بتربوو ژ ۳۰۰ملگم/لتر(WHO ..،2006)

ریژا ئوکسیجینا توێنەر کێمبوو ل ناڤ ئاڤە رویا کەهشتە 6.0ملگم/لتر ل جهی پرا کەنیسێ و ل جهێن دیتر ریژا بایوکیمیاوی یا ئوکسجینێ زێدەبوو بۆ 4.1ملگم/لتر ل جهێ پرا کەنیسێ ژبەر کو پاشماوێت کشتوکالی و روژانە دچنە ناڤ ئاڤەرویادا، هەروەسا ژمارا داگیرکەرێن بەکتیری زێدەبوو بۆ ٤۳۲ خانە بۆ ۱مل ل ئاڤێ ل جهی برا کەنیسێ

پیتێت رێ نیشاندەر : **سکرێ دهوك،جورێ ئاڤێ، چاودێری تایبەتمەندیێن ئاڤێ،پاشماوێت شارستانی.**

دراسة نوعية مياه مجرى سد دهوك

الخلاصة:

يقع سد دهوك الى شمال محافظة دهوك، تعتبرمياه سد دهوك واحدة من المصادر الرئيسية التي تجهز العديد من المناطق داخل المدينة للاغراض المدنية والشرب، تنحدر مياه السد من خلال مجرى صغير وصولا الي مياه وادي دهوك داخل المدينة الذي يصب اخيرا في سد الموصل ،تضمنت الدراسة تحديد ثلاث مواقع من هذا المجرى ،الموقع الاول من اسفل نفق السد ،الثاني من منطقة جسر البورسة والثالث من منطقة جسر الكنيسة، جمعت العينات من شهر تشرين الاول ٢٠١٠ لغاية شهر شباط ٢٠١١ ، يهدف هذا البحث الى مراقبة وتحديد الخصائص الفيزيائية والكيميائية والبايولوجية وقابليتها للاستخدامات البشرية.

وصلت قيم التوصيل الكهربائي والمواد الصلبة الكلية الى ١١١٦ مايكروسيمنز / سم، ٨٩٩ ملغم/لتر وقيمة الاس الهيدروجيني سجلت ٩,١ في محطة جسر البورسة ،اشارت القيم نحو القاعدية، وسجلت قيم العسرة الكلية ٦٠٣ ملغم/لتر في محطة جسر الكنيسة، تعتبر مياه المجرى ذات قساوة حيث جاوزت ٣٠٠ ملغم / لتر (WHO,2006). انخفضت قيم الاوكسجين المذاب خلال جريان الماء للمجرى ليصل الى ٦,٠ ملغم/ لتر في محطة جسر الكنيسة وازدادت قيم المتطلب البايوكيميائي للاوكسجين خلال المجرى ليصل الى ٤,١ ملغم/ لتر في محطة جسر الكنيسة بسبب تدفق الفضلات الزراعية والمدنية،كما ان عدد مستعمرات البكتيرية ازدادت خلال المجرى ليصل الى ٤٣٢ خلية لكل مل في محطة جسر الكنيسة.

الكلمات الدالة : سد دهوك، نوعية المياه، مراقبة خصائص المياه، المتدفقات المدنية.

A HYBRID PROPOSED IMPERIALIST COMPETITIVE ALGORITHM WITH CONJUGATE GRADIENT APPROACH FOR LARGE SCALE GLOBAL OPTIMIZATION

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Abstract

This paper presents a novel hybrid imperialist competitive algorithm called ICA-CG algorithm. Such an algorithm combines the evolution ideas of the imperialist competitive algorithm and the classic optimization ideas of the conjugate gradient, based on the compensation for solving the large scale optimization. In the ICA-CG algorithm, the process of every iteration is divided into two stages. In the first stage, the randomly, rapidity and wholeness of the imperialist competitive Algorithm are used. In the second stage, one of the common optimization classical techniques, that called conjugate gradient to move imperialist countries, is used. Experimental results for five well known test problems have shown the superiority of the new ICA-CG algorithm, in large scale optimization, compared with the classical GA, ICA, PSO and ABC algorithms, with regard to the convergence of speed and quality of obtained solutions.

Keywords**:** *Large scale global Optimization, Evolutionary Algorithms, Imperialist Competitive Algorithm (ICA),Conjugate Gradient (CG) .*

1 Introduction

The global optimization problem is applicable in every field of science, engineering and business. So far, many Evolutionary Algorithms (EA) [Sarimveis 2005] and [Srinivasan 2003], have been proposed for solving the global optimization problem. Inspired by the natural evolution, EA analogizes the evolution process of biological population, which can adapt the changing environments to the finding of the optimum of the optimization problem through evolving a population of candidate solutions. Some Evolutionary Algorithms for optimization problem are: the Genetic Algorithm (GA) [Goldberg 1989], at first proposed by Holland, in 1962 [Holland 1990], Particle Swarm Optimization algorithms (PSO) that at first proposed by Kennedy and Eberhart [Kennedy 1995], in 1995.In 2007, Artificial Bee Colony (ABC) that at first proposed by Karaboga and Basturk [Karaboga 2007], and in the same year , a new algorithm which is called Imperialist Competitive Algorithm (ICA) [Atashpaz 2007] has been proposed by Atashpaz-Gargari and Lucas , that has inspired from a socio-human phenomenon.

In general, The main advantages of Evolutionary algorithms are: they do not require the objective function to be differentiable or continuous, they do not require the evaluation of gradients and they can escape from local minima.

On the other hand, the Conjugate Gradient (CG) method [Andrei 2007] is a highly efficient direct minimization approach, which is currently the method of choice in wide areas of science and engineering. In computational solid state physics, for example, the CG method is used to minimize directly the total energy of the system of electrons, which is usually a function of a very large number of variables, in small number of iterations [Patel 2000]. The key features behind the great success of the CG approach is the conjugacy property of the search directions, and periodic restart of the iterative minimization procedure each certain number of CG steps.

In this paper, we presents a novel hybrid imperialist competitive algorithm called ICA-CG algorithm that combines the evolution ideas of the imperialist competitive algorithm and classic optimization ideas of the conjugate gradient based on the compensation for solving the large scale optimization.

The paper is organized as follows: In Section 2 some related work is presented. Section 3 describes a brief description of Imperialist Competitive Algorithm (ICA). In Section 4, a brief description of Conjugate Gradient (CG). In Section 5, new approach and the motivation of the ICA-CG algorithm is presented. In Section 6, results are compared with other Evolutionary Algorithms.

2. Some Related Works:

In 2007, Atashpaz and Lucas proposed an algorithm as Imperialist Competitive Algorithm (ICA) [Atashpaz 2007] and [Atashpaz 2008], that has inspired from a socio-human phenomenon. Since 2007 attempts were performed in order to increase the efficiency of the ICA. In 2009, Zhang, Wang and Peng proposed an approach based on the concept of small probability perturbation to enhance the movement of Colonies to Imperialist [Zhang 2009]. In 2010, Faez, Bahrami and Abdechiri, proposed a new method using the chaos theory to adjust the angle of Colonies movement toward the Imperialists' position (*CICA*)[Bahrami 2010a], and in other paper at the same year, they proposed another algorithm that applies the probability density function in order to adapt the angle of colonies' movement towards imperialist's position dynamically, during iterations (AICA) [Bahrami 2010b]. In 2012, Ghodrati, ,Malakooti and Soleimani, proposed a new hybrid method using the ICA and PSO by adding independent countries for large scale[Ghodrati 2012], and in the same year , Ramazani, Lotfi and Soltani proposed a new hybrid method called HEICA which combines Evolutionary algorithm and ICA [Ramezani 2012].

3. Imperialist Competitive Algorithm (ICA)

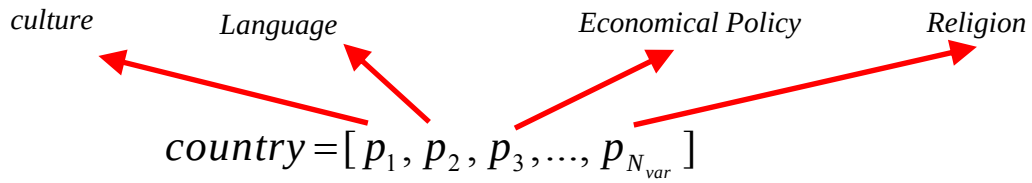
Imperialist Competitive Algorithm (ICA) is a new evolutionary algorithm in the Evolutionary Computation field based on the human's socio-political evolution. The Procedures of the ICA is presented as below:

Step 1:Creation of initial empires

The goal of optimization is to find an optimal solution in terms of the variables of the problem. We form an array of variable values to be optimized. In the GA terminology, this array is called ''chromosome", but in ICA the term ''country" is used for this array. In an N_{var}-dimentional optimisation problem, a country is a $1 * N_{var}$-array. This array is defined as following:

$$country = (p_1, p_2, p_3, \dots, p_{N_{var}}),$$

where p_i are the variables to be optimized. The variable values in the country are represented as floating point numbers. Each variable in the country can be interpreted as a socio-political characteristic of a country. From this point of view, all the algorithm does is to search for the best country that is the country with the best combination of socio-political characteristics such as culture, language, economical policy, and even religion. From optimization point of view this leads to find the optimal solution of the problem, the solution with least cost value. Figure (1) shows the interpretation of country using some of socio-political characteristics[Atashpaz 2007] and [Atashpaz 2008].



Figure(1): The candidate solutions of the problem, called country, consists of a combination of some socio-political characteristics such as culture, language and religion.

The cost of a country is found by evaluation of the cost function f at variables $(p_1, p_2, p_3, \dots, p_{N_{var}})$. So we have

$$cost = f(country) = f(p_1, p_2, p_3, \dots, p_{N_{var}}) \quad (1)$$

To start the optimization algorithm, initial countries of size $N_{country}$ is produced. We select N_{imp} of the most powerful countries to form the empires. The remaining N_{col} of the initial countries will be the colonies each of which belongs to an empire.

To form the initial empires, the colonies are divided among imperialists based on their power. That is, the initial number of colonies of an empire should be directly proportionate to its power. To proportionally divide the colonies among imperialists, the normalized cost of an imperialist is defined by

$$C_n = c_n - \max\{c_i\} \quad (2)$$

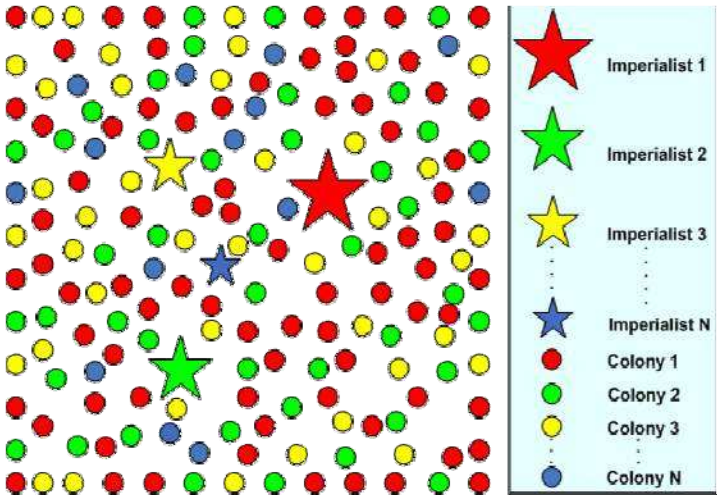
where c_n is the cost of the n[th] imperialist and C_n is its normalized cost. Having the normalized cost of all imperialists, the normalized power of each imperialist is defined by

$$p_n = \left| \frac{C_n}{\sum_{i=1}^{N_{imp}} C_i} \right| \quad (3)$$

The initial colonies are divided among empires based on their power. Then the initial number of colonies of the nth empire will be

$$N.C\_n = round\{p\_n * N\_col\} \quad (4)$$

where $N.C_n$ is the initial number of colonies of the n[th] empire and N_{col} is the total number of initial colonies. To divide the colonies, $N.C_n$of the colonies are randomly chosen and given to the n[th] imperialist. These colonies along with the nth imperialist form the nth empire. Figure (2) shows the initial empires. As shown in figure (2), bigger empires have greater number of colonies while weaker ones have less. In this figure imperialist 1 has formed the most powerful empire and consequently has the greatest number of colonies [Atashpaz 2007] and [Atashpaz 2008].



Figure(2): Generating the initial empires: The more colonies an imperialist possess, the bigger is its relevant (☆) mark.

Step2. Assimilation: movement of colonies toward the imperialist

Pursuing assimilation policy, the imperialist states tried to absorb their colonies and make them a part of themselves. More precisely, the imperialist states made their colonies to move toward themselves along different socio-political axis such as culture, language and religion. In the ICA, this process is modeled by moving all of the colonies toward the imperialist along different optimization axis. Figure (3) shows this movement. Considering a 2-dimensional optimization problem, in this figure the colony is absorbed by the imperialist in the culture and language axes. Then colony will get closer to the imperialist in these axes. Continuation of assimilation will cause all the colonies to be fully assimilated into the imperialist.

In the ICA, the assimilation policy is modeled by moving all the colonies toward the imperialist. This movement is shown in figure (3) in which a colony moves toward the imperialist by *x* units. The new position of colony is shown in a darker color. The direction of the movement is the vector from the colony to the imperialist.

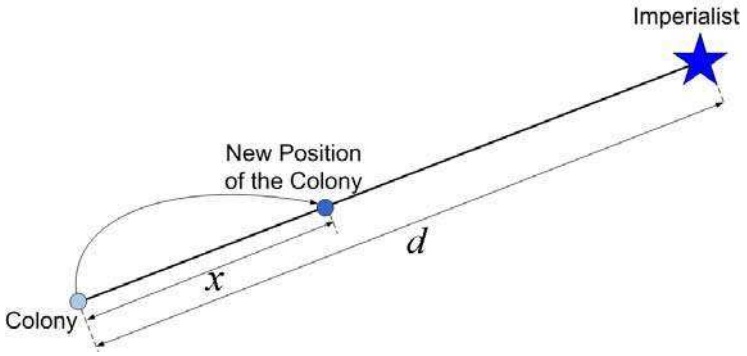


Figure (3):. Movement of colonies toward their relevant imperialist

In this figure x is a random variable with uniform (or any proper) distribution. Then

$$x \sim U(0, \beta * d) \qquad (5)$$

where β is a number greater than 1 and d is the distance between the colony and the imperialist state. β> 1 causes the colonies to get closer to the imperia list state from both sides.

Assimilating the colonies by the imperialist states did not result in direct movement of the colonies toward the imperialist. That is, the direction of movement is not necessarily the vector from colony to the imperialist. To model this fact and to increase the ability of searching more area around the imperialist, a random amount of deviation is added to the direction of movement. Figure (4) shows the new direction. In this figure θ is a parameter with uniform (or any proper) distribution. Then

$$\theta \sim U(-\gamma, \gamma) \qquad (6)$$

where γ is a parameter that adjusts the deviation from the original direction. Nevertheless the values of β and γ are arbitrary, in most of implementations a value of about 2 for β and about π/4 (Rad) for γ results in good convergence of countries to the global minimum [Atashpaz 2007] and [Atashpaz 2008].

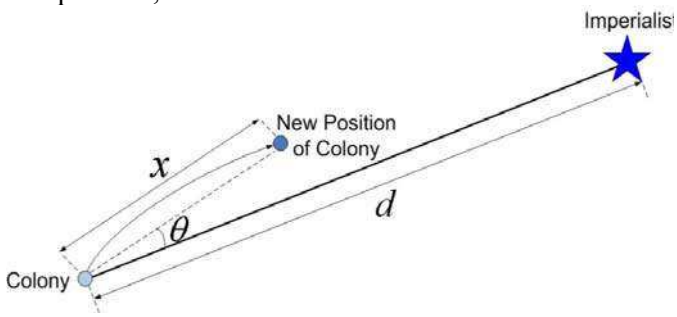


Figure (4): Movement of colonies toward their relevant imperialist in a randomly deviated direction.

Step 3. Revolution; a sudden change in socio-political characteristics of a country

Revolution is a fundamental change in power or organizational structures that takes place in a relatively short period of time. In the terminology of ICA, revolution causes a country to suddenly change its socio-political characteristics. That is, instead of being assimilated by an imperialist, the colony randomly changes its position in the socio-political axis. Figure (5) shows the revolution in Culture-Language axis. The revolution increases the exploration of the algorithm and prevents the early convergence of countries to local minimums. The revolution rate in the algorithm indicates the percentage of colonies in each colony which will randomly change their position. A very high value of revolution decreases the exploitation power of algorithm and can reduce its convergence rate. In general, the revolution rate is 0.3. That is 30 percent of colonies in the empires change their positions randomly [Atashpaz 2007] and [Atashpaz 2008].

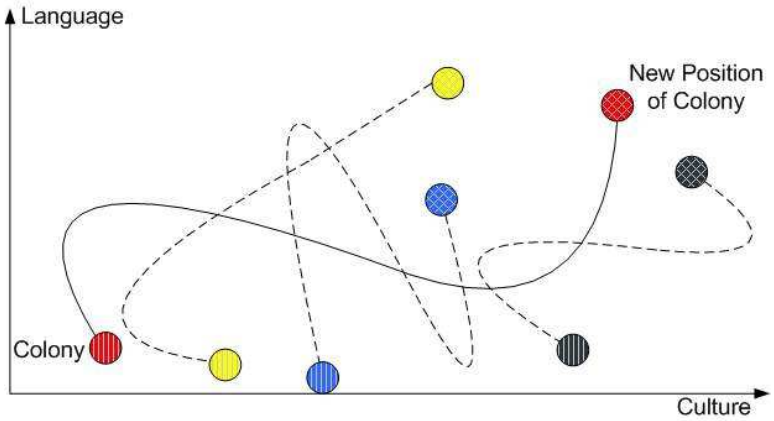
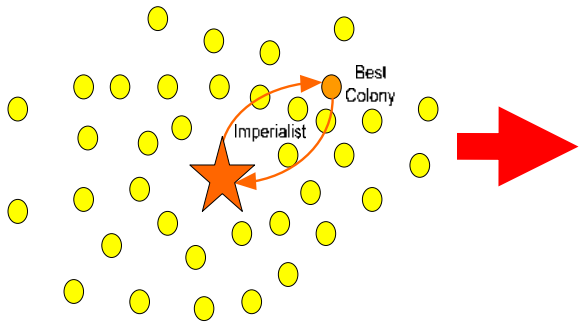


Figure (5): Revolution; a sudden change in socio-political characteristics of a country.

Step 4. Exchanging positions of the imperialist and a colony

While moving toward the imperialist, a colony might reach to a position with lower cost than the imperialist. In this case, the imperialist and the colony change their positions. Then the algorithm will continue by the imperialist in the new position and the colonies will be assimilated by the imperialist in its new position. Figure(6a) depicts the position exchange between a colony and the imperialist. In this figure the best colony of the empire is shown in a darker color. This colony has a lower cost than the imperialist. Figure (6b) shows the empire after exchanging the position of the imperialist and the colony [Atashpaz 2007] and [Atashpaz 2008].



Figure(6a): Exchanging the positions of a colony

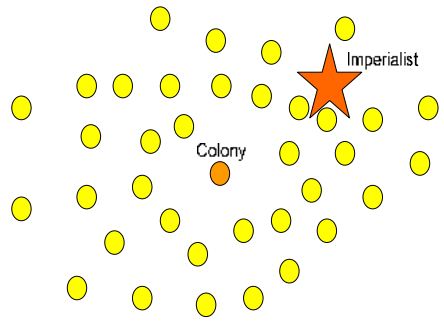


Figure (6b): The entire empire after position exchange and the imperialist

Step 5.Total power of an empire

Total power of an empire is mainly affected by the power of imperialist country. However the power of the colonies of an empire has an effect, albeit negligible, on the total power of that empire. This fact is modeled by defining the total cost of an empire by

$$T.C_n = cost(imperialist) + \varepsilon * mean\{cost(colonies\ of\ empire)\} \qquad (7)$$

Where $T.C_n$ is the total cost of the nth empire and ε is a positive small number. A little value for ε causes the total power of the empire to be determined by just the imperialist and increasing it will increase to the role of the colonies in determining the total power of an empire. The value of 0.1 for n has shown good results in most of the implementations [Atashpaz 2007] and [Atashpaz 2008].

Step 6. Imperialistic competition

All empires try to take the possession of colonies of other empires and control them. The imperialistic competition gradually brings about a decrease in the power of weaker empires and an increase in the power of more powerful ones. The imperialistic competition is modeled by just picking some (usually one) of the weakest colonies of the weakest empire and making a competition among all empires to possess these (this) colonies. Figure (7) shows a big picture of

the modeled imperialistic competition. Based on their total power, in this competition, each of empires will have a likelihood of taking possession of the mentioned colonies. In other words, these colonies will not definitely be possessed by the most powerful empires, but these empires will be more likely to possess them.

To start the competition, first a colony of the weakest empire is chosen and then the possession probability of each empire is found. The possession probability P_P is proportionate to the total power of the empire. The normalized total cost of an empire is simply obtained by

$$N.T.C_n = T.C_n - max\{T.C_n\} \quad (8)$$

Where, $T.C_n$ and $N.T.C_n$ are the total cost and the normalized total cost of nth empire, respectively. Having the normalized total cost, the possession probability of each empire is given by

$$p_n = \left| \frac{N.T.C_n}{\sum_{i=1}^{N_{imp}} N.T.C_n} \right| \quad (9)$$

To divide the mentioned colonies among empires vector P is formed as following

$$P = [p_{p_1}, p_{p_2}, p_{p_3}, \dots, p_{p_{N_{imp}}}] \quad (10)$$

Then the vector R with the same size as P whose elements are uniformly distributed random numbers is created,

$$R = \left[r_1, r_2, r_3, \dots, r_{N_{imp}}\right], r_1, r_2, r_3, \dots, r_{N_{imp}} \sim U(0,1) \quad (11)$$

Then vector D is formed by subtracting R from P

$$D = P - R = \left[D_1, D_2, D_3, \dots, D_{N_{imp}}\right] = \left[p_1 - r_1, p_2 - r_2, p_3 - r_3, \dots, p_{N_{imp}} - r_{N_{imp}}\right] \quad (12)$$

Referring to vector D the mentioned colony (colonies) is handed to an empire whose relevant index in D is maximized.

The process of selecting an empire is similar to the roulette wheel process which is used in selecting parents in GA. But this method of selection is much faster than the conventional roulette wheel. Because it is not required to calculate the cumulative distribution function and the selection is based on only the values of probabilities. Hence, the process of selecting the empires can solely substitute the roulette wheel in GA and increase its execution speed [Atashpaz 2007] and [Atashpaz 2008].

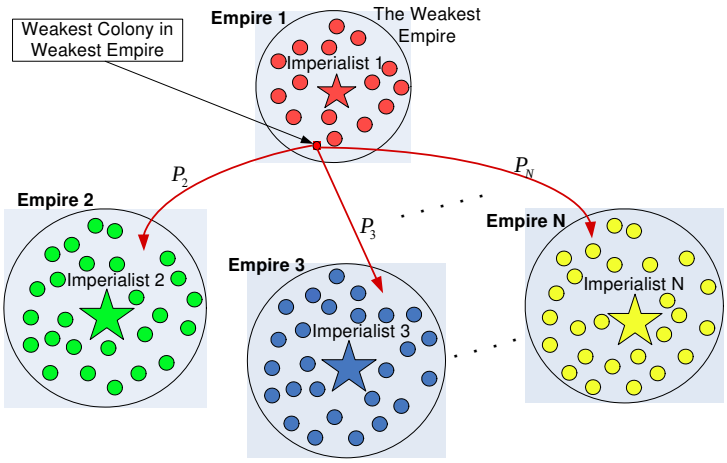


Figure (7): Imperialistic competition: The more powerful an empire is, the more likely it will possess the weakest colony of the weakest empire.

4. Conjugate Gradient Algorithm:

Conjugate gradient (CG) methods represent an important class of unconstrained optimization algorithm. The main advantages of the CG methods are its low memory requirements, its convergence speed and its poses a quadratic termination property in which the method is able to locate the minimize of quadratic function in a known finite number of iterations [Andrei 2007].

A nonlinear conjugate gradient method generates a sequence x_k, k is integer number, $k \geq 0$. Starting from an initial point x_0, the value of x_k calculate by the following equation:

$$x_{k+1} = x_k + \lambda_k d_k \qquad (13)$$

where the positive step size $\lambda_k > 0$ is obtained by a line search, and the directions d_k are generated as:

$$d_k = -g_k + \beta d_{k-1} \qquad (14)$$

where $d_0 = -g_0$, the value of β is determine according to the algorithm of Conjugate Gradient (CG), and its known as a conjugate gradient parameter, $s_k = x_{k+1} - x_k$ and $g_k = \nabla f(x_k) = f'(x_k)$, consider $\|.\|$ is the Euclidean norm and $y_k = g_{k+1} - g_k$. The termination conditions for the conjugate gradient line search are often based on some version of the Wolfe conditions. The standard Wolfe conditions:

$$f(x_k + \lambda_k d_k) - f(x_k) \leq \rho \lambda_k g_k^T d_k, \qquad (15)$$

$$g(x_k + \lambda_k d_k)^T d_k \geq \sigma g_k^T d_k, \qquad (16)$$

where d_k is a descent search direction and $0 < \rho \leq \sigma < 1$, where β_k is defined by one of the following formulas:

$$\beta_k^{(HS)} = \frac{y_k^T g_{k+1}}{y_k^T d_k} (Hestenese\ and\ Stiefel) \qquad (17)$$

$$\beta_k^{(FR)} = \frac{g_{k+1}^T g_{k+1}}{g_k^T g_k} (Fletcher\ and\ \mathrm{Re}eves) \qquad (18)$$

$$\beta_k^{(PRP)} = \frac{y_k^T g_{k+1}}{g_k^T g_k} (Polak - Ribiere\ ;and\ Polyak\ ) \qquad (19)$$

$$\beta_k^{(CD)} = -\frac{g_{k+1}^T g_{k+1}}{g_k^T d_k} (Conjugatedescent\ ) \qquad (20)$$

$$\beta_k^{(LS)} = -\frac{y_k^T g_{k+1}}{g_k^T d_k} (Liu\ and\ Stoery\ ) \qquad (21)$$

$$\beta_k^{(DY)} = \frac{g_{k+1}^T g_{k+1}}{y_k^T s_k} (Dai\ and\ Yuan\ ) \qquad (22)$$

Outlines of the CG methods:

Step1: Start with an arbitrary initial point $\boldsymbol{X_1}$.

Step 2: Set the first search direction $d_1 = -g_1 = -\nabla f(X_1)$

Step 3: Find the point $\mathbf{X}_2$ according to the relation

$$\boldsymbol{X_2 = X_1 + \lambda_1 d_1}$$

where $\boldsymbol{\lambda_1}$ is the optimal step length in the direction $\boldsymbol{d_1}$. Set $k = 2$ and go to the next step.

Step 4 Find $g_k = -\nabla f(X_k)$, and set

$$\boldsymbol{d_k = -g_k + \beta d_{k-1}}$$

where β is the conjugancy coefficient equal to $\frac{\|\boldsymbol{g_k}\|^2}{\|\boldsymbol{g_{k-1}}\|^2}$.

Step5: Compute the optimum step length $\boldsymbol{\lambda_k}$in the direction d_k, and find the new point

$$\boldsymbol{X_{k+1} = X_k + \lambda_k d_k}$$

Step6: Test for the optimality of the point$\boldsymbol{X_{k+1}}$. If $\boldsymbol{X_{k+1}}$ is optimum, stop the process. Otherwise, set the value of $k = k + 1$ and go to step 4 [Andrei 2007].

5. Hybrid ICA with CG Algorithm:

In this Section, we proposed a new hybrid ICA with conjugate gradient algorithm (CG), called ICA-CG algorithm. In the proposed hybrid algorithm, the process in every iteration is divided into two stages. In the first stage, we use the randomly, rapidity and wholeness of the imperialist competitive Algorithm. In the second stage we used FR-CG algorithm to move imperialist countries.

The important property in evolutionary algorithms is to find the best solution without needing to calculate the derivatives, while the classical methods of optimization especially conjugate gradient method, we need to calculate the derivatives because the solution moves in the negative gradient direction ($d_k = -g_k$) and therefore, we suggest here, to further improve the efficiency of classical ICA is to use calculations derivatives instead of using derivatives to avoid the loss of one of the most important properties of evolutionary algorithms.

The gradient direction g_k can be substituted by

$$\frac{f(x + \Delta x) - f(x)}{\Delta x} \qquad (23)$$

where Δx is a small positive perturbation (i.e. 1.00E-05).

The revolution rate (P_r) in the algorithm indicates the percentage of colonies in each colony which will randomly change their position. A very high value of revolution rate

decreases the exploitation power of algorithm and can reduce its convergence rate. In our algorithms the revolution rate is not constant number as original ICA, but its depending on the number of variables (N_{var}). The experimental results shows that the best revolution rate is shown in Table (1).

Table (1):represent the best revolution rate for ICA-CG

N_{var}	Revolution rate
1-10	0.001-0.01
11-20	0.01-0.05
21-50	0.05-0.08
51-100	0.08-0.1
101-1000	0.1-0.3
more than 1000	0.3-0.4

Moreover, It is worth stressing again that no explicit calculations of the true gradient are performed, and the main purpose of this work is to provide an improved ICA method. In this algorithm when CG approach make bad imperialist, we back to the old imperialist before CG approach. Figure(8) shows the flowchart of the proposed algorithm .The Procedures of the ICA-CG is presented as below:

Procedure ICA-CG
Step 1: Initializing parameters;
Step 2:
2.1 Define the optimization problem;
2.2:Generate some random countries;
2.3:Select the most powerful countries as empires;
2.4:Randomly allocate remain countries to different empires equality;
Step 3:Decade loop k=k+1 % ICA-CG operators%
Step 4:For i=1,2,…,N_{imp} do %Imperialist Competitive Algorithm%
4.1:Assimilate colonies toward their imperialist;
4.2:Countries revolution;
4.3:Exchange imperialist with best colony if is necessary;
4.4:Calculate total cost of empires;
4.5:Imperialistic competition;
4.6:Eliminate the powerless empires;
Step 5:For i=1,2,…,N_{imp} do %Conjugate Gradient%
5.1: If k=1 then $d_k=-g_k$, Otherwise $d_k=-g_k+\beta_{k-1}d_{k-1}$
5.2:Calculate the new imperialist as $x_{k+1}=x_k+\lambda d_k$, where λ is a step size which is equal to 0.001
Step 6: Terminating Criterion Control; Repeat Steps 3-6 until a terminating criterion is satisfied;

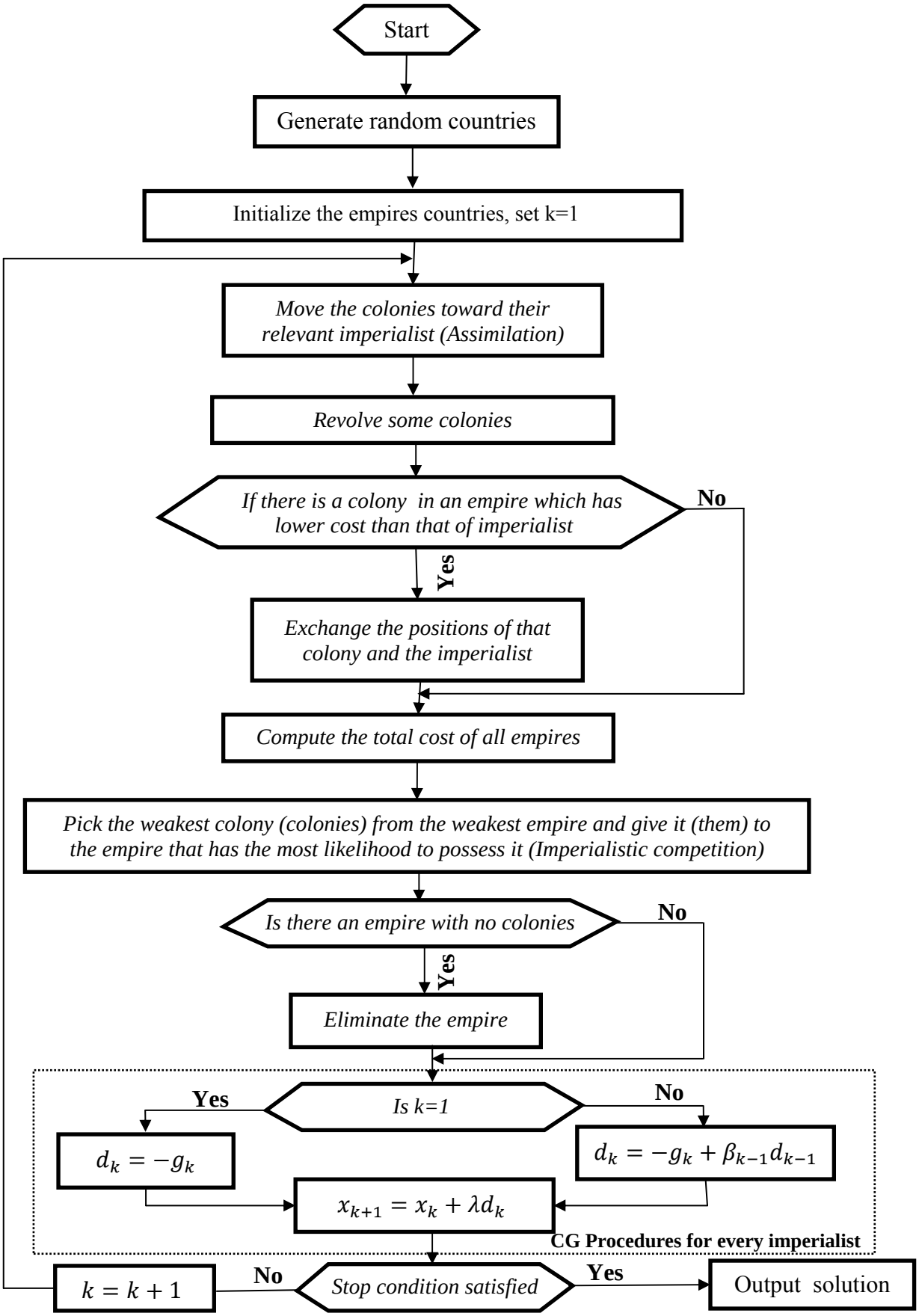


Figure (8): Flowchart of the proposed ICA-CG algorithm

5. Numerical Results

The proposed ICA-CG is tested using 5 benchmark functions [Andrei 2008]. For comparison, GA, PSO and ABC [Karaboga 2007] are also executed on these 5 functions. The parameter settings of ICA-CG algorithm are described as follows: Assimilation coefficients are set to 2.0, Revolutionary rates are set as table (1). Table (2) shows the details of test functions . The algorithm is conducted 20 runs for each test function.

Table (2): Benchmark functions (F1-F5)

Benchmarks	Function	Range
F1(Sphere)	$\sum_{i=1}^{n} x_i^2$	$[-5.12,5.12]$
F2(Rastrigin)	$\sum_{i=1}^{n} (x_i^2 - 10\cos(2\pi x_i) + 10)$	$[-5.12,5.12]$
F3(Griewank)	$1 + \sum_{i=1}^{n} (\frac{x_i^2}{4000}) - \prod_{i=1}^{n} \left(\cos\left(\frac{x_i}{\sqrt{i}}\right)\right)$	$[-600,600]$
F4(Ackly)	$-20\exp\left(-0.2\sqrt{\frac{1}{n}\sum_{i=1}^{n} x_i^2}\right) - \exp\left(\frac{1}{n}\sum_{i=1}^{n}\cos(2\pi x_i)\right) + 20 - e$	$[-30,30]$
F5(SumSquares)	$\sum_{i=1}^{n} i^2 x_i^2$	$[-1,1]$

In Table (3), the performance of ICA-CG algorithm is compared with GA,ICA, PSO and ABC [13] for high dimensional problem .

Table (3): Comparing the Performance ICA-CG with GA , PSO and ABC (high dimensions)

F.	N_{var}	Pop	Gen	P_r	GA	ICA	PSO	ABC	9
F1	100	500	1000	0.1	9.70E-01	1.71E+02	1.99E+00	8.59E−05	**2.02E-16**
	500	600	1500	0.25	2.02E+01	2.94E+03	3.90E+03	2.26E+02	**6.49E-10**
	1000	800	2000	0.3	6.40E+01	6.72E+03	8.00E+03	1.46E+03	**5.29E-09**
F2	100	500	1000	0.1	5.81E+01	9.17E+02	6.05E+02	5.39E+01	**2.46E-05**
	500	600	1500	0.25	1.01E+03	7.14E+03	8.59E+03	1.93E+03	**1.31E+02**
	1000	800	2000	0.3	2.97E+03	1.54E+04	1.74E+04	6.05E+03	**5.08E+02**
F3	100	500	1000	0.1	1.32E-02	7.56E+02	5.24E−01	9.04E−03	**3.95E-08**
	500	600	1500	0.25	6.19E+01	1.03E+04	5.10E+01	9.48E+02	**2.20E-01**
	1000	800	2000	0.3	1.14E+03	2.38E+04	2.98E+02	4.86E+03	**1.50E+00**
F4	100	500	1000	0.1	2.74E+00	1.90E+00	3.37E+00	2.12E+00	**1.24E-07**
	500	600	1500	0.25	9.01E+01	3.49E+01	2.09E+01	1.49E+01	**1.96E-05**
	1000	800	2000	0.3	7.80E+03	7.26E+03	1.74E+04	1.77E+01	**2.80E-05**
F5	100	500	1000	0.1	8.69E+01	1.75E+04	1.39E+02	1.11E−03	**4.35E-08**
	500	600	1500	0.25	9.02E+02	7.41E+06	1.12E+07	5.23E+05	**9.96E+00**
	1000	800	2000	0.3	3.39E+04	7.46E+07	9.50E+07	1.84E+08	**9.77E+02**

Conclusion

This paper proposes hybrid algorithm consisting of ICA and CG. The performance of this algorithm is evaluated using various test functions. These functions are set of well-known multi-dimensional benchmark functions. The simulations indicate that the proposed algorithm has outstanding performance in speed of convergence and precision of the solution for global optimization. This is meaning that it has the capability to come up with non-differentiable objective functions with a multitude number of local optima through reasonable time limit. The results show the efficiency and capabilities of the new hybrid algorithm in finding the optimum. Also, the performance of such algorithm is better than other algorithms such as GA, ICA, PSO and ABC. Indeed, the performance achieved of this study is quite satisfactory and promising for all test functions.

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خوارزمية تنافسية امبريالية مقترحة مهجنة مع خوارزمية الاتجاه المترافق لحل مسائل الامثلية ذات القياس الكبير

الخلاصة :

في هذا البحث تم اقتراح خوارزمية مهجنة جديدة تدعى اختصاراً خوارزمية $ICA-CG$، وذلك بربط الافكار التطورية للخوارزمية التنافسية الامبريالية مع افكار الامثلية التقليدية متمثلة في خوارزمية الاتجاه المترافق لحل مسائل الأمثلية الكبيرة، في هذه الخوارزمية المقترحة تقسم العملية في كل تكرار إلى مرحلتين، في المرحلة الاولى تم استخدام العشوائية والسرعة لخوارزمية الـ ICA، وفي المرحلة الثانية تم استخدام واحدة من تقنيات الامثلية التقليدية المعروفة والتي تدعى خوارزمية $FR-CG$ لتحريك البلدان الامبريالية. وقد أظهرت التجارب العددية لخمسة مسائل اختبار قياسية تفوق خوارزمية $ICA-CG$ الجديدة في حل مسائل الأمثلية الكبيرة مقارنة بالخوارزميات التقليدية كالخوارزمية الجينية GA وخوارزمية أمثلة أسراب الطيور PSO وخوارزمية مستعمرات النحل ABC من ناحية سرعة التقارب ونوعية الحلول المكتسبة.

NUMERICAL SOLUTION OF KAWAHARA EQUATION USING NEURAL NETWORK

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Abstract

An artificial neural network technique is proposed in this research to solve the well-known partial differential equations of the types: Kawahara and modified Kawahara equations. The mathematical model of the equation was developed with the help of artificial neural networks. The construction requires imposing certain constrains on the values of the input, bias and output weights, and on the attribution of certain roles of each aforementioned parameters. The results obtained from the proposed technique were very accurate, simple and convenient. Moreover, the comparison between the approximated solutions and the exact one has done. This comparison found them in a good agreement with each other due to of superior properties of the Neural Network.

Keyword*: Kawahara Equation, Modified Kawahara Equation, Artificial Neural Network.*

1. INTRODUCTION

In the past several decades, the investigation of solutions for nonlinear equations has played an important role in the study of nonlinear physical phenomena.

The Kawahara equation was first proposed by Kawahara in 1972, as a model equation describing solitary wave propagation in media Kawahara T.1972. The Kawahara and Generalized Kawahara equation occurs in the theory of magneto-acoustic waves in plasma D. T. Pham, E. Koc, A. Ghanbarzadeh and S. Otri 2006. and in the theory of shallow water waves with surface tension. However, recently some researchers have used artificial neural network (ANN) to solve differential equationsin numerical calculation to overcome such limitations A. Junaid, M. A. Z. Raja, and I. M. Qureshi2009.H. A . Jalab, Rabha W. Ibrahim, Shayma A. Murad, Amera I. Melhum, and S. B.Hadid 2012. Many researchers have spent a great deal of effort to compute the solution of the Kawahara equation using various numerical methods. Recently, In V. G. Gupta and S. Gupta2010, the homotopy perturbation method (HPM) is employed to obtain approximate analytical solutions of the Kawahara equation and generalized Kawahara equation. M. Kurulay2012 applied the homotopy analysis to solve the modified Kawahara equation. Exp-function method used to study the exact solution for the Kawahara equation in N. A. Kudryashov2010.

Sh. Bahzadi 2011,solved the Kawahara equation by using the Adomain's decomposition method, modified Adomian's decomposition method, variational iteration method, modified variational iteration method, homotopy perturbation method, modified homotopy perturbation method and homotopy analysis method.

2. THE MODEL PROBLEMS

The analysis presented in this paper is based on the Kawahara equation (Sirendaoreji , 2004) :

$$u_t + uu_x + u_{xxx} - u_{xxxxx} = 0 \qquad (1)$$

with the initial condition: $u(x,0) = f(x)$

The exact solution of the above equation is:

$$u(x,t) = \frac{-72}{169} + \frac{105}{169}\, sech^4 \frac{1}{2\sqrt{13}}\left(x + \frac{36}{169}t\right)$$

For example

while we consider modified Kawahara equation **(** Sirendaoreji , 2004) , is:

$$u_t + u^2u_x + pu_{xxx} + qu_{xxxxx} = 0 \qquad (2)$$

where p,q are nonzero real constants, with the initial condition $u(x,0) = f(x)$
The exact solution is given for modified Kawahara equation by **(** Sirendaoreji , 2004):

$$u(x,t) = \frac{3p}{\sqrt{-10q}} sech^2(K(x-ct))$$

With $c = \frac{25q-4p^2}{25q}$ and $K = \frac{1}{2}\sqrt{\frac{-p}{5q}}$

In this paper we apply an artificial neural network to estimate the solutions of the Kawahara equation (1) and the for modified Kawahara equation (2).

3. ARTIFICIAL NEURAL NETWORK (ANN)

Neural networks are computational models of the biological brain. Like the brain, a neural network comprises a large number of interconnected neurons. Each neuron is capable of performing only simple computation M. Ghalambaz, A.R. Noghrehabadi, M.A. Behrang, E. Assareh, A. Ghanbarzadeh and N. Hedayat 2011, D. T. Pham, E. Koc, A. Ghanbarzadeh and S. Otri2006. Anyhow, the architecture of an artificial neuron is simpler than a biological neuron. ANNs are constructed in layer connected to one or more hidden layers where the factual processing is performance through weighted connections. Each neuron in the hidden layer joins to all neurons in the output layer. The results of the processing are acquired from the output layer. Learning in ANNs is achieved through particular training algorithms which are expanded in accordance with the learning laws, assumed to simulate the learning mechanisms of biological system A. Malek and R. S. Beidokhti 2006. However, as an assembly of neurons, a neural network can learn to perform complex tasks including pattern recognition, system identification, trend prediction, function approximation, and process control D. T. Pham, E. Koc, A. Ghanbarzadeh and S. Otri 2006. Multi-layer Perceptron (MLPs) are perhaps the most common type of feed forward networks D. T. Pham and X. Liu1995, A. S. Yilmaz and Z. Ozer2009 .

In this work, a standard back-propagation neural network (NN) is used to estimate the exact solution for the given fractional equation. The network consists of three layers; the first layer consists of neurons that are responsible for input data vectors into the neural network. The second layer is a hidden layer. This layer allows neural network to perform the error reduction, which is necessary to successfully achieve the desired output. The final layer is the output layer which is

Determined by the size of the set of desired outputs, which represent the estimated exact solution. Each possible output is represented by a separate neuron. There is one output from neural network. The neural network structure is shown in Fig. 1.

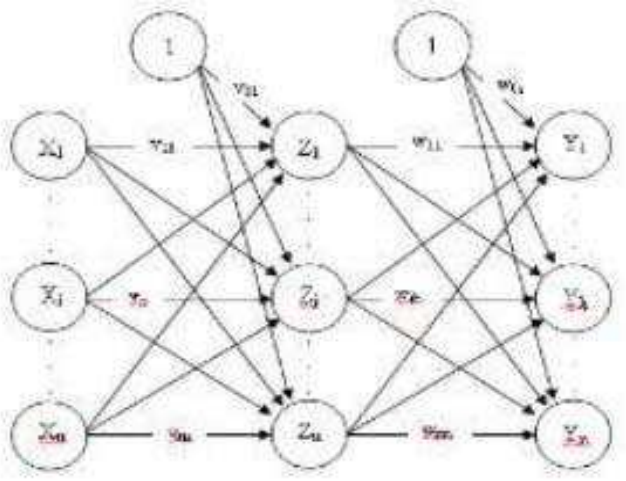

Fig.1 Neural Network structure

Each neuron j (Fig. 2) in the hidden layer sums up its input signals x_i after weighting them with the strengths of the respective connections w_{ij} from the input layer and adding the bias b_i to them, and computes its output η_i as a function g of the sum

$$\eta_i = g(\sum w_{ij} x_i + b_i) \qquad (3)$$

where η_i is each neuron output and g can be a simple threshold function or a sigmoid.

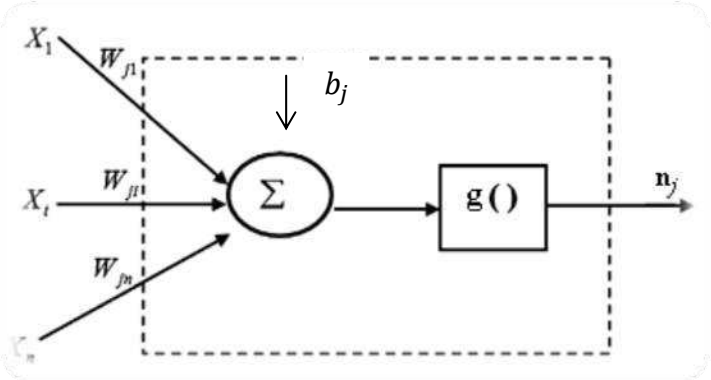

Fig.2 Details of a neuron

The general training algorithm for BPN is as follows

Step0. Initialize weights.
(Set to small random values)
Step1. While stopping, condition is false(output meet the goal), do Step 2-9.
Step2. For each training pair, do Steps 3-8.

Feedforward:

Step3. Each input unit $(X_i,\ i = 1 \dots n)$ receive input signal X_i and broadcasts this signal to all units in the layer above (the hidden units).
Step4. Each hidden unit $(Z_j,\ j = 1 \dots p)$ sums it

$$z\_in_j = v_{oi} + \sum_{i=1}^{n} x_i\, v_{ij} \qquad (4)$$

Applies its activation function to compute its output signal.

$$Z_i = f(z\_in_j) \qquad (5)$$

and send this signal to all units in the layer above (output units)
Step5. Each output unit $(Y_k,\ k = 1 \dots m)$ sums its weighted input signals

$$y\_in_k = w_{0k} + \sum_{j=1}^{p} z_j\, w_{jk} \qquad (6)$$

And applies its activation function to compute its output signal

$$y_k = f(y\_in_k) \qquad (7)$$

Backpropagation of error:

Step6. Each output unit $(Y_k,\ k = 1 \dots m)$ receives a target pattern corresponding to the input training pattern, computes its error information term.

$$\delta_k = (t_k - y_k) f'(y\_ in_k) \qquad (8)$$

Calculates its weight correction term (used to update w_{jk} later).

$$\Delta w_{ik} = \alpha \delta_k z_j \qquad (9)$$

Calculates its bias correction term (used to update w_{0k} later).

$$\Delta w_{0k} = \alpha \delta_k \qquad (10)$$

and sends δ_k to units in the layer below.
Step7. Each hidden unit $(Z_j,\ j = 1 \dots p)$ sums its delta inputs (from units in the layer above).

$$\delta\_in_j = w_{0k} + \sum_{k=1}^{p} \delta_k\, w_{jk} \qquad (11)$$

Multiplies by the derivative of its activation function to calculate its error information term

$$\delta_j = \delta\_in_j\, f'(z\_in_j) \qquad (12)$$

Calculate its weight correction term (used to update v_{ij} later).

$$\Delta v_{ij} = \alpha \delta_j x_i \qquad (13)$$

and calculate its bias correction term (used to update v_{0j} later)

$$\Delta v_{0j} = \alpha \delta_j (14)$$

Update weights and biases:

Step8. Each output unit $(Y_k,\ k = 1 \dots m)$ update its bias and weights $(j = 0 \dots p)$:

$$w_{jk}(new) = w_{jk}(old) + \Delta w_{jk} \qquad (15)$$

Each hidden unit $(Z_j,\ j = 1 \dots p)$ updates its bias and weights$(i = 0 \dots n)$:

$$v_{ij}(new) = v_{ij}(old) + \Delta v_{ij} \qquad (16)$$

Step9. Test stopping condition. [13].

4. Artificial Neural Network Methodology for Kawahara Equation

Neural networks generally provide improved performance with the normalized data. The use of original data as input to neural network may cause a convergence problem. All the data sets were therefore, transformed into values between -1 and 1 through dividing the difference of actual and minimum values by the difference of maximum and minimum values subtracted by 1. At the end of each algorithm, outputs were renormalized into the original data format for achieving the desired result.

Training goal for the networks was set to 10^{-5}. Finding appropriate architecture needs trial and error method.

Networks were trained for a fixed number of epochs. By this way, we found that two neurons for two hidden layer produce good result. Comparison the result of ANN and exact solution is shown in Fig. 3.

1. Create an architecture consists of two input nodes in the input layer, two hidden nodes in two hidden layers, one output node in the output layer. Assign the nodes to each layer.
2. Initialize the weights and bias to random values.
3. Initialize the network parameters.
4. Train the network with initialized parameters, and with sigmoid activation function.
5. Repeat the process until the maximum epochs are reached or the desired output is identified or the minimum gradient is reached.

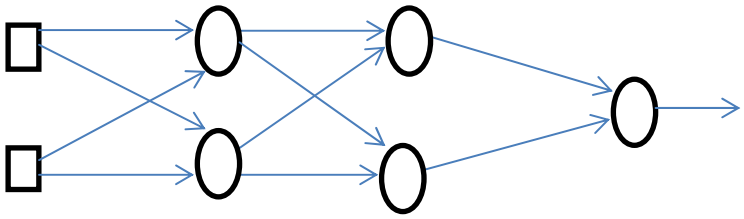

Fig. 3 Neural Network structure for kawahara equation

EXAMPLE 1. To show the procedure, we will examine Kawahara equation

$$u_t + uu_x + u_{xxx} - u_{xxxxx} = 0$$

With initial condition

$$u(x,0) = \frac{-72}{169} + \frac{105}{169} sech^4\left(\frac{1}{2\sqrt{13}}x\right)$$

The exact solution is

$$u(x,t) = \frac{-72}{169} + \frac{105}{169} sech^4 \frac{1}{2\sqrt{13}}\left(x + \frac{36}{169}t\right)$$

for computing work: Comparison between the exact solution and the artificial neural network solution will be given in the following (Table 1):

Table 1: Comparison of results for the solutions of example 1 where $x \in (0,2)$, $t \in (0,1)$

Kawahara Equation			
T	x	ANN Solution	Exact Solution
0.1	0.1	0.194818435344505	0.194915325641
0.1	0.2	0.194134854223350	0.194097364623
0.1	0.3	0.192782703802976	0.192805204738
0.1	0.4	0.191039486048669	0.191042568078
0.1	0.5	0.188833513582170	0.188812796301
0.1	0.6	0.186115595708242	0.186121568496
0.1	0.7	0.182957732770161	0.182977236589
0.1	0.8	0.179393463430124	0.179389100063
0.1	0.9	0.175383881161043	0.175364895242
0.1	1	0.170903532930394	0.170914456985
0.1	1.1	0.166008717725511	0.166051894564
0.1	1.2	0.160830163626410	0.160786354712
0.1	1.3	0.155484418862169	0.155513423562
0.1	1.4	0.149951486630059	0.149108256974
0.1	1.5	0.144126956820884	0.142723459831
0.1	1.6	0.138580203948702	0.1359952886166
0.2	0.6	0.185481153647747	0.1854893572
0.2	0.7	0.182265474605305	0.182249967
0.2	0.8	0.178640125774316	0.1785673955
0.2	0.9	0.174550793023224	0.174451295
0.2	1	0.169973265199086	0.1699123841

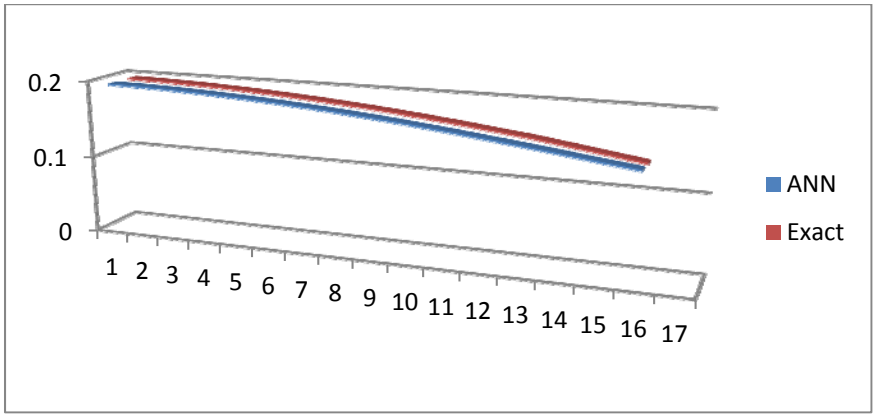

Fig3. Neural Network estimated solution for Example (1)

EXAMPLE 2. We have taken $p = 0.001$ *and* $q = -1$, then the modified Kawahara equation is

$$u_t + u^2 u_x + 0.001 u_{xxx} - u_{xxxxx} = 0$$

with initial condition $u(x,0) = \frac{0.003}{\sqrt{10}} sech^2(0.00707106x)$

The exact solution of modified Kawahara equation as follow:

$$u(x,t) = \frac{0.003}{\sqrt{10}} sech^2(0.00707106(x - 1.00000016t))$$

for computational work, , will be given in Table 2

Table 2. Comparison of results for the solution of example2 $= 0.001$ *and* $q = -1$, $x \in R$, $t \in (0,1)$

Modified Kawahara Equation			
t	x	ANN Solution	Exact Solution
0.02	-5	0.000947424133242253	0.0009474889415215798
0.04	-2.5	0.000948316063770784	0.0009483773375906261
0.06	0.0	0.000948590405265281	0.0009486831272874861
0.08	2.5	0.000948390995627245	0.0009484055588301033
0.10	5	0.000947705508736422	0.0009475453146340136
0.12	7.5	0.000946215002182919	0.0009461045077496208
0.14	10	0.000944010475646170	0.0009440866708438895
0.16	12.5	0.000941399960100077	0.0009414967378249370
0.18	15	0.000938351483701271	0.0009383410182732446
0.20	17.5	0.000934608800307114	0.0009346271649062464
0.22	20	0.000930050731366098	0.0009303641343629944
0.06	-1.25	0.000948730474574495	0.0009486031388265163
0.07	0	0.000948664477167285	0.0009486831272874861
0.08	1.25	0.000948514966415799	0.0009486172537859130
0.09	2.5	0.000948270673895690	0.0009484055588301033
0.10	3.75	0.000947888953488847	0.0009480481725713649
0.11	5	0.000947320814390300	0.0009475453146340136
0.12	6.25	0.000946548126945595	0.0009468972938286883

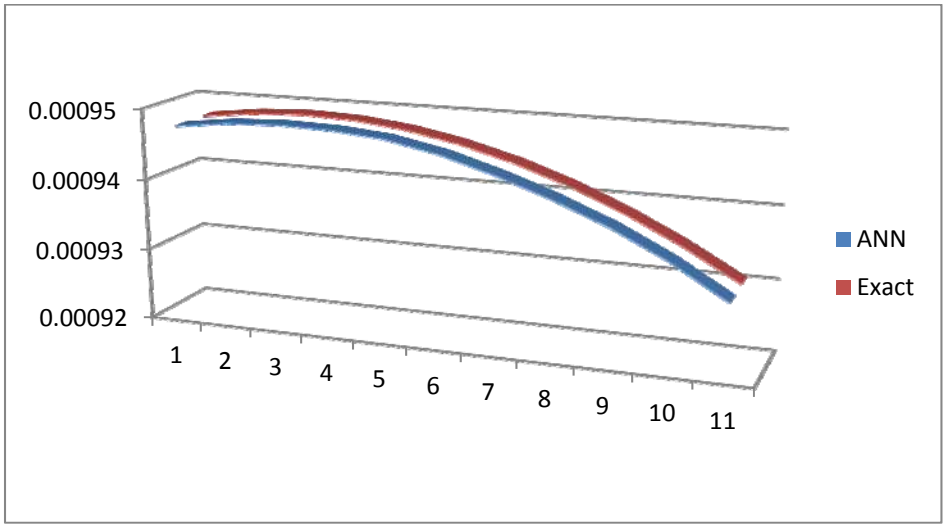

Fig4. Neural Network estimated solution for Example (2)

5. CONCLUSION

This research indicated that the artificial neural network (ANN) technique can play an important role to estimate the solutions of the well-known equations: Kawahara and modified Kawahara equation. Therefore, the research has shown that the estimated solution by using ANN technique is approximately to the exact solution. Although the obtained results have acceptable accuracy, the proposed technique can be improved by increasing the number of training data, and can be minimized the error and reduced the differences. Further, the parallel processing property of neural network had reduced the computational time which makes this method better than the conventional methods.

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الملخص

تناولنا فى هذا البحث , دراسة الحل لبعض المعادلات التفاضلية الجزئية من نوع معادلة كاهوارا ومعادلة كاهوارا المحسنة باستخدام الشبكات العصبية. النتائج التى تم الحصول عليها من هذه الطريقة جدا متقاربة من الحل الحقيقى .

كورتى

د ڤێ ڤەكولینێدا هاتە خواندن شیكار كردن هندە ك هاوكێشەیێن جیاكاریێن بشك ژلای هاوكیشا كەهوارە وهاوكیشە كەهوارە یاجیتر ب رێكا تورین نیورین. ئانجمت كفتینا بن دەست ڤالك نیزیكن اش شیكاركردن درست.

PERIODIC SOLUTION FOR NONLINEAR SYSTEM OF DIFFERENTIAL EQUATIONS DEPENDING ON THE GAMMA DISTRIBUTION

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Abstract

In this paper we study the periodic solution of nonlinear system of differential equations depending on the gamma distribution by using the numerical analytic method to investigate periodic solutions of ordinary differential equation which given by Samoilenko A. M. .These investigations lead us to improving and extending this method. Also we expand the results gained by Samoilenko A. M. to change the periodic system of nonlinear differential equations to periodic system of nonlinear differential equations depending the on gamma distribution.

Keyword: *Periodic Solution, Differential Equations, Gamma Distribution, Numerical Analytic.*

1.Introduction.

They are many subjects in physics and technology using mathematical methods that depends on the linear and nonlinear differential equations, and it become clear that the existence of periodic solutions and its algorithm structure form important problems, to present time where many of studies and researches [1,4,5,6] dedicates for treatment the autonomous and non-autonomous periodic systems and specially with differential equations.

Numerical-analytic method [1,2,3,5] owing to the great possibilities of exploiting computers are becoming versatile means of the finding and approximate construction of periodic solutions of differential equations. Samoilenko [4] assumes the numerical-analytic method to study the periodic solutions for ordinary differential equations and its algorithm structure and this method include uniformly sequences of periodic functions and the results of that study is using of the periodic solutions on wide range in the difference of new processes industry and technology as in [3,5,6].

Samoilenko[4] has been used the numerical-analytic method to studythe periodic solution for nonlinear system of differential equation which has the form:

$$\frac{dx}{dt} = f(t,x) \quad \cdots (1.1)$$

where

$x \in D$, $all\ real\ t\ \ and\ \ D\ is\ the\ closure\ of\ bounded\ domain\ and\ connected\ in\ R^n$.

In this study we have employed the numerical-analytic method of Samoilenko .[4] to investigate the existence and approximation of periodic solution for nonlinear system of differential equations which depends on th gamma distribution. The study of such differential equations leads to improving and extending Samoilenko method [4].

Thus, the differential equations which depends on the gamma distribution that we have introduced in this study, becomes more general and detailed than those introduced by Samoilenko .[4]. The study is considered a theoretical one, however, the results that we have got, many several applications in the physical field as well as mathematical problems.

Consider the following system of differential equations which has the form

$$\frac{dx}{dt} = f(t, \gamma(t,\alpha), x) \ \cdots (1.2)$$

where

$x \in D$, D *is the closure of bounded domain and connected in* R^n. The vector function $f(t,\gamma(t,\alpha),x)$ is defined on the domain:

$$(t,\gamma(t,\alpha),x) \in R^1 \times [0,T] \times D = (-\infty,\infty) \times [0,T] \times D \qquad \cdots (1.3)$$

Continuous for all variables, periodic in t of period T and satisfies the inequalities:

$$|f(t,\gamma(t,\alpha),x)| \le MM_\alpha \ , \qquad M_\alpha > 0 \qquad \cdots (1.4)$$

$$|f(t,\gamma(t,\alpha),x_1) - f(t,\gamma(t,\alpha),x_2)| \le M_\alpha(K|x_1 - x_2|) \qquad \cdots (1.5)$$

for all $t \in R^1$ and $x, x_1, x_2 \in D$, where $M = (M_1, M_2, \cdots, M_n)$is a positive constant vectors and the gamma distribution is defined as

$$\left.\begin{aligned} &\gamma(t,\alpha) = \frac{t^{\alpha-1}e^{-t}}{\Gamma(\alpha)} \quad , \ \alpha > 0 \, , \\ &\text{where} \qquad T < \left(\frac{\Gamma(\alpha+1)}{(\alpha+1)}\right)^{\frac{1}{\alpha}} . \end{aligned}\right\} \qquad \cdots (1.6)$$

We define the non-empty sets as follows:

$$D_{\gamma f} = D - MM_\alpha \frac{T}{2} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots . (1.7)$$

Furthermore, we suppose that the greatest eigen value λ_{max} of the matrix $\Lambda = M_\alpha \, K\frac{T}{2}$ does not exceed unity, i.e.

$$\lambda_{max}(\Lambda) < 1 \qquad \cdots (1.8)$$

Lemma 1.1[4]. Let $f(t)$ be a continuous vector function defined on the interval $[0,T]$, then

$$\left| \int_0^t (f(s) - \frac{1}{T}\int_0^T f(s)ds)ds \right| \le \alpha(t) \max_{t\in[0,T]} |f(t)|$$

where $\alpha(t) = 2t(1-\frac{t}{T})$. (For the proof see [4]) .

By using Lemma 1.1 , we can state and prove the following Lemma.

Lemma 1.2. Suppose that the function $\gamma(t,\alpha)$ of gamma distribution is continuous on the interval $[0,T]$. Then

$$\left| \int_0^t (\gamma(s,\alpha) - \frac{1}{T}\int_0^T \gamma(s,\alpha)ds)ds \right| \le M_\alpha \alpha(t)$$

is hold for all values of α . where $M_\alpha = \max_{t\in[0,T]}|\gamma(t,\alpha)|$.

Proof. Taking

$$\left| \int_0^t (\gamma(s,\alpha) - \frac{1}{T}\int_0^T \gamma(s,\alpha)ds)ds \right| \le \left(1-\frac{t}{T}\right)\int_t^T |\lambda(s,\alpha)|ds + \frac{t}{T}\int_t^T |\lambda(s,\alpha)| \, ds$$

$$= \left(1-\frac{t}{T}\right)\int_0^t \frac{T^{\alpha-1}e^{-t}}{\Gamma(\alpha)}ds + \frac{t}{T}\int_t^T \frac{T^{\alpha-1}e^{-t}}{\Gamma(\alpha)}ds$$

$$\le \frac{T^{\alpha-1}e^{-t}}{\Gamma(\alpha)}\left[\left(1-\frac{t}{T}\right)t + \frac{t}{T}(T-t)\right]$$

$$= \alpha(t) \ M_\alpha$$

so that

$$\left| \int_0^t (\gamma(s,\alpha) - \frac{1}{T}\int_0^T \gamma(s,\alpha)ds)ds \right| \leq \alpha(t)M_\alpha \cdots (1.10)$$

for all $t \in [0,T]$ and $\alpha(t) \leq \frac{T}{2}$.

2. Approximate solution.

The investigation of periodic approximate solution of (1.2) is formulated by the following theorem.

Theorem 2.1. If the system (1.2) satisfy the inequalities (1.4),(1.5) and conditions (1.6),(1.7) has a periodic solution $x = x(t,\gamma(t,\alpha),x_0)$, then the sequence of functions

$$x_{m+1}(t,\gamma(t,\alpha),x_0) = x_0 + \int_0^t [f(s,\gamma(s,\alpha),x_m(s,\gamma(s,\alpha),x_0) - \frac{1}{T}\int_0^T (f(s,\gamma(s,\alpha),x_m(s,\gamma(s,\alpha),x_0)ds]ds \ldots . (2.1)$$

with

$x_0(t,\gamma(t,\alpha),x_0) = x_0$, $\quad m = 0,1,2,\cdots$

is periodic in t of period T, and uniformly convergent to the limit function $x^0(t,\gamma(t,\alpha),x_0)$ as $m \to \infty$ in the domain

$(t,\gamma(t,\alpha),x_0) \in R^1 \times [0,T] \times D_{\gamma f} \cdots (2.2)$

periodic in t of period T and satisfying the system of integral equations

$$x(t,\gamma(t,\alpha),x_0) = x_0 + \int_0^t [f(s,\gamma(s,\alpha),x(s,\gamma(s,\alpha),x_0) - \frac{1}{T}\int_0^T (f(s,\gamma(s,\alpha),x(s,\gamma(s,\alpha),x_0)ds]ds \cdots (2.3)$$

which is a unique solution of the problem (1.2) provided that:

$$|x^0(t,\gamma(t,\alpha),x_0) - x_0| \leq M\, M_\alpha\, \alpha(t) \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \quad \cdots (2.4)$$

and

$$|x^0(t,\gamma(t,\alpha),x_0) - x_m(t,\gamma(t,\alpha),x_0)| \leq \Lambda^m(E-\Lambda)^{-1}M\, M_\alpha\, \alpha(t) \cdots (2.5)$$

for all $m \geq 1$ and $t \in R^1$, where E is the identity matrix.

Proof:

By Lemma 1.1, 1.2 and using (2.1), when $m = 0$, we get:

$$|x_1(t,\gamma(t,\alpha),x_0) - x_0| \leq (1-\frac{t}{T})\int_0^t |f(s,\gamma(s,\alpha),x_0, \int_s^{s+T} g(\tau,\gamma(\tau,\alpha),x_0)\, d\tau)|ds$$

$$+\frac{t}{T}\int_{t}^{T}|f(s,\gamma(s,\alpha),x_0,\int_{s}^{s+T} g(\tau,\gamma(\tau,\alpha),x_0)\,d\tau)|ds$$

$$\leq (1-\frac{t}{T})\int_{0}^{t} M\,M_\alpha\,ds+\frac{t}{T}\int_{t}^{T} M\,M_\alpha\,ds$$

$$=M\,M_\alpha\,[(1-\frac{t}{T})t+\frac{t}{T}(T-t)]$$

$$=2t(1-\tfrac{t}{T})\;M\,M_\alpha$$

$$=\alpha(t)\,M\,M_\alpha$$

So that

$|x_1(t,\gamma(t,\alpha),x_0)-x_0|\leq M\,M_\alpha\alpha(t)\cdots(2.6)$

i.e. $x_1(t,\gamma(t,\alpha),x_0)\in D$, for all $t\in R^1, x_0\in D_{\gamma f}$.

Thus by mathematical induction, we find that:

$$|x_m(t,\gamma(t,\alpha),x_0)-x_0|\leq M\,M_\alpha\alpha(t)\cdots(2.8)$$

for all $t\in R^1$ and $x_0\in D_{\gamma f}$.

i.e. $x_m(t,\gamma(t,\alpha),x_0)\in D$, for all $t\in R^1$ and $x_0\in D_{\gamma f}$.

We claim that the sequence of functions (2.1) is uniformly convergent on the domain (2.2).

By using the Lemmas 1.1,1.2 and putting $m=1$ in (2.1), we have:

$$|x_2(t,\gamma(t,\alpha),x_0)-x_1(t,\gamma(t,\alpha),x_0)|\leq K[(1-\frac{t}{T})\int_{0}^{t}|\gamma(s,\alpha)||x_1(s,\gamma(s,\alpha),x_0)-x_0|ds$$

$$+\frac{t}{T}\int_{t}^{T}|\gamma(s,\alpha)|\,|x_1(s,\gamma(s,\alpha),x_0)-x_0|ds]$$

$$\leq M_\alpha\,M\,M_\alpha K\frac{T}{2}\alpha(t)$$

$$|x_2(t,\gamma(t,\alpha),x_0)-x_1(t,\gamma(t,\alpha),x_0)|\leq M\,{M_\alpha}^2K\frac{T}{2}\alpha(t)\cdots(2.9)$$

Suppose that the following inequality is true

$$|x_m(t,\gamma(t,\alpha),x_0)-x_{m-1}(t,\gamma(t,\alpha),x_0)|\leq M\,{M_\alpha}^m[K\frac{T}{2}]^{m-1}\alpha(t)$$

$$\cdots(2.10)$$

for all $m\geq 1$.

Now, we shall prove the following:

$$|x_{m+1}(t,\gamma(t,\alpha),x_0)-x_m(t,\gamma(t,\alpha),x_0)|$$

$$\leq K[\left(1-\frac{t}{T}\right)\int_{0}^{t} M_\alpha\;|x_m(s,\gamma(s,\alpha),x_0)-x_{m-1}(s,\gamma(t,\alpha),x_0)|ds$$

$$+\frac{t}{T}\int_{t}^{T} M_\alpha\,|x_m(s,\gamma(s,\alpha),x_0)-x_{m-1}(s,\gamma(t,\alpha),x_0)|ds]\leq$$

$$\leq\left(1-\frac{t}{T}\right)\int_{0}^{t} M_\alpha\,M\,{M_\alpha}^m\left[K\frac{T}{2}\right]^{m-1}\;\alpha(s)ds$$

$$+\frac{t}{T}\int_{t}^{T} M_\alpha\, M\, {M_\alpha}^m \left[K\frac{T}{2}\right]^{m-1} \alpha(s)ds$$

$$= M\, M_\alpha \left[M_\alpha K\frac{T}{2}\right]^m \alpha(t)$$

and hence

$$|x_{m+1}(t,\gamma(t,\alpha),x_0) - x_m(t,\gamma(t,\alpha),x_0)| \le MM_\alpha \left[M_\alpha K\frac{T}{2}\right]^m \alpha(t) \cdots (2.11)$$

for all $m \ge 0$.

From (2.11) we conclude that for any$k \ge 1$, we have the inequality

$$|x_{m+k}(t,\gamma(t,\alpha),x_0) - x_m(t,\gamma(t,\alpha),x_0)| \le \sum_{i=0}^{k-1} \Lambda^{m+i}\, M\, M_\alpha\ \alpha(t)$$

such that

$|x_{m+k}(t,\gamma(t,\alpha),x_0) - x_m(t,\gamma(t,\alpha),x_0)|$

$\le \sum_{i=0}^{\infty}|x_{m+1+i}(t,\gamma(t,\alpha),x_0) - x_{m+i}(t,\gamma(t,\alpha),x_0)|$

$$\le \sum_{i=0}^{\infty} MM_\alpha\alpha(t)\,\Lambda^{m+1+i}$$

$$\le M\, M_\alpha\, \alpha(t)\,\Lambda^m \sum_{i=0}^{\infty} \Lambda^{i+1}$$

$$\le M\, M_\alpha\, \alpha(t)\,\Lambda^m\,(\mathrm{E}-\Lambda)^{-1}$$

so that

$$|x_{m+k}(t,\gamma(t,\alpha),x_0) - x_m(t,\gamma(t,\alpha),x_0)| \le\ \Lambda^m\,(\mathrm{E}-\Lambda)^{-1} M\, M_\alpha\, \alpha(t) \quad \cdots (2.12)$$

for all $k \ge 1$.

From (2.12) and the condition (1.9), we find that:

$$\lim_{m\to\infty} \Lambda^m = 0 \quad \cdots (2.13)$$

Relations (2.12) and (2.13) insures the uniform convergence of the sequence of functions (2.1) on the domain (2.2).

Let

$$\lim_{m\to\infty} x_m(t,\gamma(t,\alpha),x_0) = x^0(t,\gamma(t,\alpha),x_0) \cdots (2.14)$$

Since the sequence of functions (2.2) is periodic in t of period T, then the limiting function $x^0(t,\gamma(t,\alpha),x_0)$ is also periodic in t of period T.

Moreover, by Lemmas 1.1,1.2 and inequality (2.12) the inequalities (2.4) and (2.5) are holds.

Finally, we have to show that $x(t,\gamma(t,\alpha),x_0)$ is a unique solution of the system (1.1).Assume that $r(t,\gamma(t,\alpha),x_0)$ is another solution of the system (1.1), i.e.

$$r(t,\gamma(t,\alpha),x_0) = x_0 + \int_0^t [f(s,\gamma(s,\alpha),r(s,\gamma(s,\alpha),x_0) - \frac{1}{T}\int_0^T (f(s,\gamma(s,\alpha),r(s,\gamma(s,\alpha),x_0)ds]ds, \quad \ldots (2.15)$$

Now, we prove that $x(t,\gamma(t,\alpha),x_0) = r(t,\gamma(t,\alpha),x_0)$ for all $x_0 \in D_{\gamma f}$ and to do this, we need to derive the following inequality:

$$|r(t,\gamma(t,\alpha),x_0) - x(t,\gamma(t,\alpha),x_0)| \le\ \Lambda^m\,(\mathrm{E}-\Lambda)^{-1}M^*\, M_\alpha\, \alpha(t) \quad \cdots (2.16)$$

where $M^* = max_{x_0 \in D_{\gamma f}} \left| f\left(t, r(t, \gamma(t,\alpha), x_0), x(t, \gamma(t,\alpha), x_0)\right)\right|$.
Suppose that (2.15) is true for $m = k$, i.e.
$|r(t, \gamma(t,\alpha), x_0) - x(t, \gamma(t,\alpha), x_0)| \leq \Lambda^k \, (\mathrm{E} - \Lambda)^{-1} M^* \, M_\alpha \, \alpha(t)$
then

$$|r(t, \gamma(t,\alpha), x_0) - x(t, \gamma(t,\alpha), x_0)|$$
$$\leq K[\left(1 - \frac{t}{T}\right) \int_0^t M_\alpha \, |r(s, \gamma(s,\alpha), x_0) - x(s, \gamma(s,\alpha), x_0)| ds + \frac{t}{T} \int_t^T M_\alpha \, |r(s, \gamma(s,\alpha), x_0) - x(s, \gamma(s,\alpha), x_0)| ds]$$
$$\leq K[\left(1 - \frac{t}{T}\right) \int_0^t M_\alpha \, \Lambda^k (E - \Lambda)^{-1} M^* M_\alpha \alpha(s) ds + \frac{t}{T} \int_t^T M_\alpha \, \Lambda^k (E - \Lambda)^{-1} M^* M_\alpha \alpha(s) ds]$$
$$= \Lambda^{k+1} (E - \Lambda)^{-1} M^* M_\alpha \alpha(t).$$

By induction, inequality (2.16) is true for $m = 0,1,2, \cdots$.
Thus from (2.14) and (2.16), we have:
$\lim_{m \to \infty} |r(t, \gamma(t,\alpha), x_0) - x_m(t, \gamma(t,\alpha), x_0)| = 0$

and hence
$\lim_{m \to \infty} x_m(t, \gamma(t,\alpha), x_0) = r(t, \gamma(t,\alpha), x_0)$
By using the relation (2.14), we get:
$x(t, \gamma(t,\alpha), x_0) = r(t, \gamma(t,\alpha), x_0)$
i.e. $x(t, \gamma(t,\alpha), x_0)$ is a unique solution of (1.1) on the domain (1.2). ■

3. Existence of solution.

The problem of existence of a periodic solution of period T of the system (1.1) is uniquely connected with the existence of zeros of the function $\Delta(0, \gamma(0,\alpha), x_0)$ which has the form:

$\Delta(0, \gamma(0,\alpha), x_0) = \frac{1}{T} \int_0^T f(t, \gamma(t,\alpha), x^0(t, \gamma(t,\alpha), x_0) dt \ldots\ldots\ldots\ldots \cdots (3.1)$
where $x^0(t, \gamma(t,\alpha), x_0)$ is the limiting function of the sequence of functions (2.1).
The function (3.1) can be determined only approximately, say by computing the following functions:

$\Delta_{\mathrm{m}}(0, \gamma(0,\alpha), x_0) = \frac{1}{T} \int_0^T f(t, \gamma(t,\alpha), x_m(t, \gamma(t,\alpha), x_0) dt \ldots\ldots\ldots \cdots (3.2)$
$m = 0,1,2, \cdots.$

Now, we prove the following theorem.

Theorem3.1. If all assumptions and conditions of theorem 2.1 are hold, then the inequality:
$|\Delta(0, \gamma(0,\alpha), x_0) - \Delta_{\mathrm{m}}(0, \gamma(0,\alpha), x_0)| \leq \Lambda^{m+1} (E - \Lambda)^{-1} \, M \, M_\alpha \cdots (3.3)$
will be satisfied for all $m \geq 0, x_0 \in D_{\gamma f}$.

Proof. From relations (3.1) and (3.2), the estimate

$$|\Delta(0, \gamma(0,\alpha), x_0) - \Delta_{\mathrm{m}}(0, \gamma(0,\alpha), x_0)|$$
$$\leq \frac{K}{T} \int_0^T |\gamma(t,\alpha)| \, |x^0(t, \gamma(t,\alpha), x_0) - x_m(t, \gamma(t,\alpha), x_0)| dt$$

$$\leq \frac{K}{T}\int_0^T M_\alpha\, \Lambda^m (E-\Lambda)^{-1} M\, M_\alpha\, \alpha(t)dt$$
$$= \Lambda^{m+1}(E-\Lambda)^{-1} M\, M_\alpha$$

Thus the inequality (3.2) is hold for all $m \geq 0$.

Next, we prove the following theorem taking into account that the inequality (3.3) will be satisfied for all $m \geq 0$.

Theorem 3.2. If the system (1.1) satisfies the following condition

(i) The sequence of functions (3.2) has an isolated singular point $x_0 = x^0$, $\Delta_{\mathrm{m}}(0, \gamma(0,\alpha), x^0) \equiv 0$, for all $t \in R^1$.

(ii) The index of this point is nonzero;

(iii) There exists a closed convex domain D_γ^* belonging to domain $D_{\gamma f}$ and possessing a unique singular point x^0 such that on its boundary $\Gamma_{D_\gamma^*}$ the following inequality hold

$$inf_{x_0 \in \Gamma_{D_\gamma^*}} \|\Delta_{\mathrm{m}}(t, \gamma(t,\alpha), x_0)\| \geq \|\Lambda^m (E-\Lambda)^{-1} M\, M_\alpha\| \cdots \quad (3.4)$$

Where $x_0 \in \Gamma_{D_\gamma^*}$ for all $m \geq 0$. Then the system (1.1) has a periodic solution $x = x(t, \gamma(t,\alpha), x_0)$ for which $x(0, \gamma(0,\alpha), x_0)$ belongs to the domain D_γ^* .

Proof. By using the inequality (3.1) the proof is similar to that of theorem 7.1[4].

Remark 3.1.[4]. When $R^n = R^1$, i.e. when $x_0$is a scalar, the existence of solution can be strengthens by giving up the requirement that the singular point shout be isolated, thus we have

Theorem3.3. Let the system of nonlinear differential equations (1.1) are defined on the interval $[a, b]$. Suppose that for $m \geq 0$, the function $\Delta_{\mathrm{m}}(0, \gamma(0,\alpha), x_0)$ defined according to formula (3.2) satisfies the inequalities:

$$\left.\begin{array}{ll} \min\limits_{a+h \leq x_0 \leq b-h} \|\Delta_{\mathrm{m}}(t, \gamma(t,\alpha), x_0)\| \leq -\sigma_m & ; \\ \max\limits_{a+h \leq x_0 \leq b-h} \|\Delta_{\mathrm{m}}(t, \gamma(t,\alpha), x_0)\| \geq \sigma_m & . \end{array}\right\} \quad \cdots (3.5)$$

Then the system (1.1) has a periodic solution $x = x(t, \gamma(t,\alpha)x_0)$ for which $x_0 \in [a+h, b-h]$, where $h = \|M\, M_\alpha\| \frac{T}{2}$ and $\sigma_m = \|\Lambda^{m+1}(E-\Lambda)^{-1} M\, M_\alpha\|$.

Proof. Let x_1 and x_2 be any two points on the interval $[a, b]$ such that:

$$\left.\begin{array}{ll} \Delta_{\mathrm{m}}(0, \gamma(0,\alpha), x_1) = \min\limits_{a+h \leq x_0 \leq b-h} \Delta_{\mathrm{m}}(0, \gamma(0,\alpha), x_0) & ; \\ \Delta_{\mathrm{m}}(0, \gamma(0,\alpha), x_2) = \max\limits_{a+h \leq x_0 \leq b-h} \Delta_{\mathrm{m}}(0, \gamma(0,\alpha), x_0) & . \end{array}\right\} \quad \cdots (3.6)$$

From the inequalities (3.3) and (3.5), we have

$$\left.\begin{array}{l} \Delta(0, \gamma(0,\alpha), x_1) = \Delta_{\mathrm{m}}(0, \gamma(0,\alpha), x_1) + [\Delta(0, \gamma(0,\alpha), x_1) - \Delta_{\mathrm{m}}(0, \gamma(0,\alpha), x_1)] \\ \Delta(0, \gamma(0,\alpha), x_2) = \Delta_{\mathrm{m}}(0, \gamma(0,\alpha), x_2) + [\Delta(0, \gamma(0,\alpha), x_2) - \Delta_{\mathrm{m}}(0, \gamma(0,\alpha), x_2)] \end{array}\right\} \quad \cdots (3.7)$$

It follows from the inequalities (3.7) and the continuity of the function $\Delta(0, \gamma(0,\alpha), x_0)$, that there exist an isolated singular point $x^0, x^0 \in [x_1, x_2]$, such that $\Delta(0, \gamma(0,\alpha), x_0) \equiv 0$, this means that the system (1.1) has a periodic solution $x = x(t, \gamma(t,\alpha), x_0)$ for which $x_0 \in [a+h, b-h]$. ■.

Theorem3.4. If the function $\Delta(0, \gamma(0,\alpha), x_0)$ is defined by

$\Delta: D_{\gamma f} \to R^n$,

$$\Delta(0, \gamma(0,\alpha), x_0) = \frac{1}{T}\int_0^T f(t, \gamma(t,\alpha), x^0(t, \gamma(t,\alpha), x_0)dt \ldots\ldots \cdots (3.7)$$

where$x^0(t, \gamma(t,\alpha), x_0)$ is a limit of the sequence of functions (2.1). Then the following inequalities are holds

$|\Delta(0,\gamma(0,\alpha),x_0)| \leq \mathrm{M}\ \mathrm{M}_\alpha \cdots (3.8)$
and

$$|\Delta(0,\gamma(0,\alpha),{x_0}^1) - \Delta(0,\gamma(0,\alpha),{x_0}^2)| \leq \frac{2}{T}\Lambda(E-\Lambda)^{-1}M_\alpha \ \cdots (3.9)$$

for all $x_0, {x_0}^1,\ {x_0}^2 \in D_{\gamma f}$.

Proof. From the properties of the function $x^0(t,\gamma(t,\alpha),x_0)$ as in theorem 2.1; it follows that the function $\Delta(0,\gamma(0,\alpha),x_0)$ is continuous and bounded by $M\ M_\alpha$.
By using (3.7), we get:

$$|\Delta(0,\gamma(0,\alpha),{x_0}^1) - \Delta(0,\gamma(0,\alpha),{x_0}^2)| = \left|\frac{1}{T}\int_0^T f(t,\gamma(t,\alpha),x^0(t,\gamma(t,\alpha),{x_0}^1)dt - \frac{1}{T}\int_0^T [f(t,\gamma(t,\alpha),x^0(t,\gamma(t,\alpha),{x_0}^2)ds]ds\right|$$

$$\leq \frac{K}{T}\int_0^T M_\alpha\ |x^0(t,\gamma(t,\alpha),{x_0}^1) - x^0(t,\gamma(t,\alpha),{x_0}^2)|dt$$

$$\leq M_\alpha K\frac{T}{2}.\frac{2}{T}|x^0(t,\gamma(t,\alpha),{x_0}^1) - x^0(t,\gamma(t,\alpha),{x_0}^2)|dt = \frac{2}{T}\Lambda|x^0(t,\gamma(t,\alpha),{x_0}^1) - x^0(t,\gamma(t,\alpha),{x_0}^2)|$$

and hence

$$|\Delta(0,\gamma(0,\alpha),{x_0}^1) - \Delta(0,\gamma(0,\alpha),{x_0}^2)| \leq \frac{2}{T}\ \Lambda\ |x^0(t,\gamma(t,\alpha),{x_0}^1) - x^0(t,\gamma(t,\alpha),{x_0}^2)|M_\alpha \cdots (3.10)$$

where ${x_0}^1(t,\gamma(t,\alpha),x_0)$ and ${x_0}^2(t,\gamma(t,\alpha),x_0)$ are solutions of the integral equation:

$$x(t,\gamma(t,\alpha),{x_0}^k) = {x_0}^k + \int_0^t [f(s,\gamma(s,\alpha),x(s,\gamma(s,\alpha),{x_0}^k) - \frac{1}{T}\int_0^T (f(s,\gamma(s,\alpha),x(s,\gamma(s,\alpha),{x_0}^k)ds]ds \ \cdots (3.11)$$

with

${x_0}^k(t,\gamma(t,\alpha),x_0) = {x_0}^k$, $\quad k = 1,2.$

From (3.11), we have:

$$|x^0(t,\gamma(t,\alpha),{x_0}^1) - x^0(t,\gamma(t,\alpha),{x_0}^2)| \leq |{x_0}^1 - {x_0}^2| + K[\left(1-\frac{t}{T}\right)\int_0^t M_\alpha\ |x^0(s,\gamma(s,\alpha),{x_0}^1) - x^0(s,\gamma(s,\alpha),{x_0}^2)|ds + \frac{t}{T}\int_t^T M_\alpha\,|x^0(s,\gamma(s,\alpha),{x_0}^1) - x^0(s,\gamma(s,\alpha),{x_0}^2)|ds]$$

$$\leq |{x_0}^1 - {x_0}^2| + M_\alpha K\frac{T}{2}|x^0(t,\gamma(t,\alpha),{x_0}^1) - x^0(t,\gamma(t,\alpha),{x_0}^2)|\alpha(t) \leq |{x_0}^1 - {x_0}^2| + \Lambda|x^0(t,\gamma(t,\alpha),{x_0}^1) - x^0(t,\gamma(t,\alpha),{x_0}^2)|$$

thus

$|x^0(t,\gamma(t,\alpha),{x_0}^1) - x^0(t,\gamma(t,\alpha),{x_0}^2)| \leq (E-\Lambda)^{-1}|{x_0}^1 - {x_0}^2| \cdots (3.12)$
using the inequality (3.12) in (3.10), we get (3.9).

Remark 3.2.[2]. The theorem 3.4 ensure the stability solution of the system (1.1) when there is a slight change on the point x_0 accompanied with noticeable change in the function $\Delta(0, \gamma(0, \alpha), x_0)$.

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الملخص

يتضمن البحث دراسة الحل الدوري لنظم المعادلات التناضلية اللأخطية المعتمدة على توزيع كاما وذللك بأستخدام الطريقة التحليلية _العددية لدراسة الحلول الدورية للمعادلات التفاضلية المعطاه في المرجع Samoilenko .هذه الدراسة تقودنا الى تحسين وتوسيع هذه الطريقة وكذلك تم توسيع نتائج Samoilenko وذلك بتحويل المعادلات التفاضلية اللاخطية الدورية الى معادلات تفاضلية لأخطية دورية معتمدة على توزيع كاما

MEASUREMENT OF VISUAL EEG SIGNALS STIMULATED BY YELLOW COLOR PHOTONS OF SPECIFIC WAVE LENGTH AT DIFFERENT INTENSITIES

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Abstract:

Electroencephalography (EEG) is a diagnostic tool that records the electrical activity of the brain using numerous electrodes placed on various regions to the scalp, or in special cases on the cortex. The resulting traces are known as an electroencephalogram and represent so-called brainwaves.

There are many types of stimulators (physical, chemical , and mechanical)that are affect the special senses sensitive areas in the brain which are collected as electrical signals. This research is concerned with photo stimulator (yellow color with frequency of ≈ 515 THz that is transmitted by visual nerve and the generated electrical signal waves are recorded by EEG. The sample of the research consists of 18 normal vision volunteers divided equally between males and females. All precautions of wave interference or artifacts that are due to physiological or external electrical source are taken into account and minimized into the ignored level.

The results clearly showed that, the mean relative action potential amplitude recorded from O_2 for males were the same in low and higher intensities ; when intensity was increased than that recorded from O_1, and O_2 channel for females also the same at low intensities then increased with increasing intensity than that recorded from O_1.

The fitting equation of the mean relative action potential amplitude versus the intensity of O_1 and O_2 channels for males are $y = 0.053x^2-3.161x + 47.23$ with $R^2 = 0.969$ and $y = 0.044x^2- 2.619x + 39.19$ with $R^2 = 0.987$, and for females are $y = 0.046x^2 – 2.693x + 39.60$ with $R^2 = 0.894$ and $y = 0.043x^2- 2.483x + 36.59$ with $R^2 = 0.880$ respectively.

To compare the EEG electrical signals that are recorded from O_1 and O_2 channels for males and females, the mean relative action potential amplitudes are higher in males than in females for both channels. The deduced fitting curves for males O_1 channel is $y =0.046x^2 – 2.693x + 39.60$ with $R^2 = 0.894$ and for females, O_1 channel $y =0.043x^2 - 2.483x + 36.59$ with $R^2 = 0.880$ and that for male O_2 channel is $y = 0.044x^2 – 2.619x + 39.19$ with $R^2 = 0.987$ and for female O_2 channel $y = 0.05x^2 – 3.61x + 47.23$ with $R^2 = 0.969$.

Keywords: *Visual, EEG, Photons, Wave length, Brain*

Introduction:

The electroencephalogram (EEG) is an instrument that recording the electrical activity along various regions of the scalp produced by the firing of neurons action potential within the brain (Niedermeyer, 2004). It is a graphic display of the difference in voltages from two sites of brain function recorded over time. EEG can be used to predict abnormal development and aid in the evaluation of nonspecific symptoms such as behavioral disorders, anxiety, or learning disabilities. On the other hand, by the addition of the averaging computer, electrical potential recording has been extended to the whole class of evoked or event related potentials. This improving the clinicians ability to diagnose multiple sclerosis and lesions located in the optic nerve, brain stem, cerebellopontine angle, and spinal cord. Finally EEG can also be used in conjunction with other types of brain imaging (Shamsaei, 2008).

A visual evoked potential (VEP) is an evoked potential caused by a visual stimulus, that are recorded from electrodes that are placed on the back of head and observed as a reading on an electroencephalogram . These responses usually originate from the occipital cortex, the area of the brain involved in receiving and interpreting visual signals (Leslie, 2006; Vaughan,1965and Edmund,2006).

VEP has become routinely used and extremely valuable in both research and clinical evaluation of visual sensory and perceptual processing. The current research focused on EEG signals generated in the visual cortex and recorded by channels connected to O_1 and O_2 since these channels are the most sensitive channels (figure1) for specific photon's wave lengths (yellow color photons of wavelength ≈ 570 nm) at different intensities and to compare the response as EEG signals generated in males and females of the same age groups with normal Visual functions.

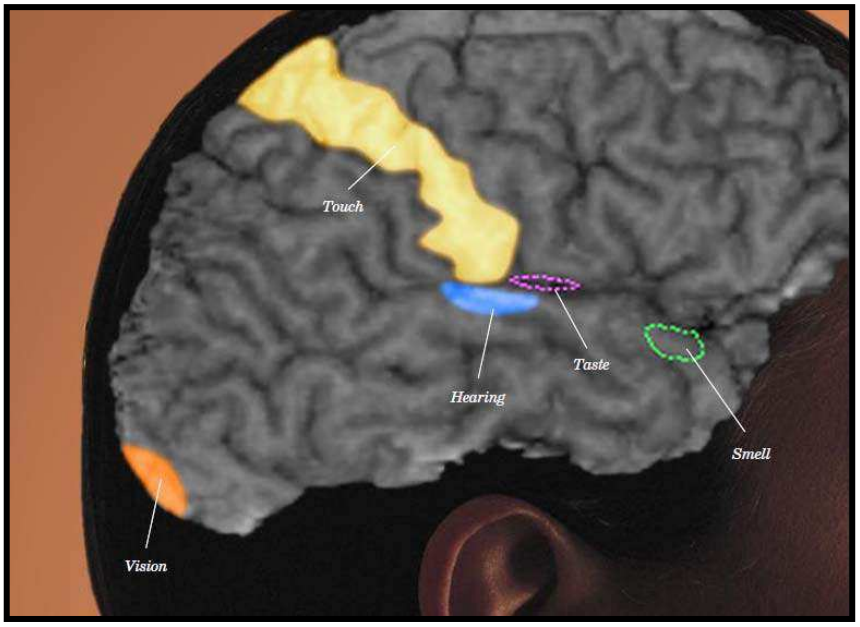

Figure 1: Showing the five senses activate separate areas of the cerebral cortex (Edmund,2006).

Materials and Methods:

1- Equipments and Tools:

EEG system used in the present study was KT88 Digital EEG and Mapping System Automatically recording EEG Unit, with 16 EEG leads and 2 ECG leads with an ability of Automatic measurement of multifunctional flash light of USB port and manual or automatic control. The International Standard Electrode placement (10/20) method was used.

The electrodes pick up the signals from the head surface, amplifiers bring the microvolt into the range where they can be digitalized accurately, and converter changes the signals from analogue to digital readings which are displayed and stored in the computer monitor system.

2- Subjects and Techniques:

Subjects 18 subjects under test were divided into two groups, nine normal males and nine females and with an age range between (25- 28) years.

Each subject was sitting on a comfortable chair; their eyes were opened and looking forward to the screen of the computer. All electrodes were fixed on the scalp through the net work connecting channels from these channels O_1 and O_2 electrodes were selected (Edmund,2006). It is worthwhile to mention that these areas are the most sensitive through which light stimulating parameter (photons of certain intensities and frequencies) are received by retina and transmitted through visual nerve as electrical signal to these symmetrical areas to be recognized. Then a stimulating power spectrum produced through which the mean relative power spectrum can be determined (the ratio of the power spectrum after exposure to that before) at different frequencies (yellow color photons of frequency $\approx$ 515 THz) and at different light intensities (26.1, 32, 33.2, and 36 lux) for subject's in a completely dark room and the eyes are opened as well as the distance between subject eye and computer screen was 2 cm.

Results and Discussion:

The results of the current study showed that the amplitude of artifacts can be quite large relative to the size of amplitude of the cortical signals of interest. This is one of the reasons why it needs a considerable experience to correctly interpret EEGs clinically. Some of the most common types of physiological artifacts include: Eye induced artifacts (includes eye blinks, eye movements and extra-ocular muscle activity), EKG (cardiac) artifacts, EMG (muscle activation)-induced artifacts, and Glossokinetic artifacts ...etc .

The physiological artifacts were controlled by asking the subject under test to stop all the voluntary activities that may generate unwanted

electrical signals that makes distortion to the real signal that we want to deduce. Other generated physiological artifacts may due to external chemical ,physical, and mechanical stimulators that affect specific sense and cause the firing of Electrical signals which interfere with the real electrical signal to be record. These signals can be minimized either by stop the cause or to be sure that there is no external stimuli.

In addition to artifacts generated by the body, many artifacts originate from the environmental. Movement by the subject, or even just settling of the electrodes, poor grounding of the EEG electrodes can cause significant 50 or 60 Hz artifact, depending on the local power system's frequency. A third source of possible interference can be the presence of an IV drip; such devices can cause rhythmic, fast, low-voltage bursts, which may be confused for spikes (Barry,1965 and Iwasaki, 2005).

The mean relative action potential amplitude recorded from O_1 and O_2 for males and females volunteers are showing in tables (1) and (2)and in figures (1) and (2). As the figures indicate, the mean relative amplitudes that are recorded from channel O_1 is different from that recorded from channel O_2 for both males and females. This difference is decreased at intensity 32 lux then increased with increasing the intensity. The fitting equation of the mean relative action potential amplitude versus the intensity of O_1 and O_2 channels for males and female were $y = 0.053x^2-3.161x + 47.23$ with $R^2 = 0.969$ and $y = 0.044x^2- 2.619x + 39.19$ with $R^2 = 0.987$, and $y = 0.046x^2 – 2.693x + 39.60$ with $R^2 = 0.894$ and $y = 0.043x^2- 2.483x + 36.59$ with $R^2 = 0.880$, respectively.

To compare between the EEG electrical signals that are recorded from O1 and O_2 channels for males and females, we found that the mean relative action potential amplitudes are the same at low intensity (26.1 Lux) and higher in male than in females for both channels at other intensities (Figures 3 and 4). The deduced fitting curve for males O_1 channel is $y =0.046x^2 – 2.693x + 39.60$ with $R^2 = 0.894$ and for females, O1 channel is $y =0.043x^2 - 2.483x + 36.59$ with $R^2 = 0.880$ and that for male O_2 channel is $y = 0.044x^2 – 2.619x + 39.19$ with $R^2 = 0.987$ and for female O_2 channel $y = 0.05x^2 – 3.61x + 47.23$ with $R^2 = 0.969$.

It can be concluded from the results of the current study that male's visual area is more sensitive for yellow color than that for females for both channels (O_1 and O_2), and both males and females are more comfortable to yellow color at an intensity of (32 Lux). This means that some intensities is more comfortable than others for same color, specially yellow color since it has been noted in Holy Quran that bright yellow color is impresses the viewers.

Table (1): The mean relative action potential recorded from O_1 and O_2 Channels of male subjects.

	Intensity (Lux)	Mean Relative action potential of O_1 Channel	Mean Relative action potential of O_2Channel
Males	26.1	1.205	1.28
	32	0.935	0.838
	33.2	1.489	1.617
	36	2.658	2.915

Table (2): The mean relative action potential recorded from O_1 and O_2 Channels of female subjects.

	Intensity (Lux)	Mean Relative action potential of O_1 Channel	Mean Relative action potential of O_2Channel
Females	26.1	1.196	1.181
	32	0.95	0.931
	33.2	2.166	2.108

36	**3.165**	**2.995**

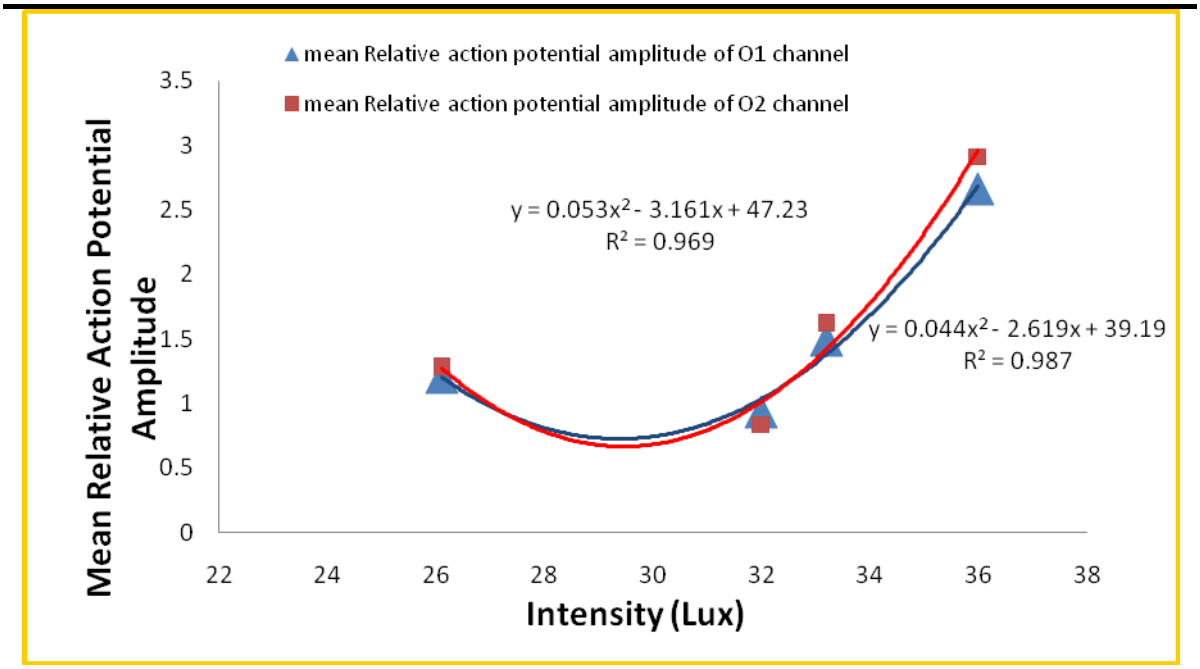

Figure (1): The relation between the intensity of yellow color (Lux) and the mean relative action potential amplitudes for males of O_1 and O_2 channels.

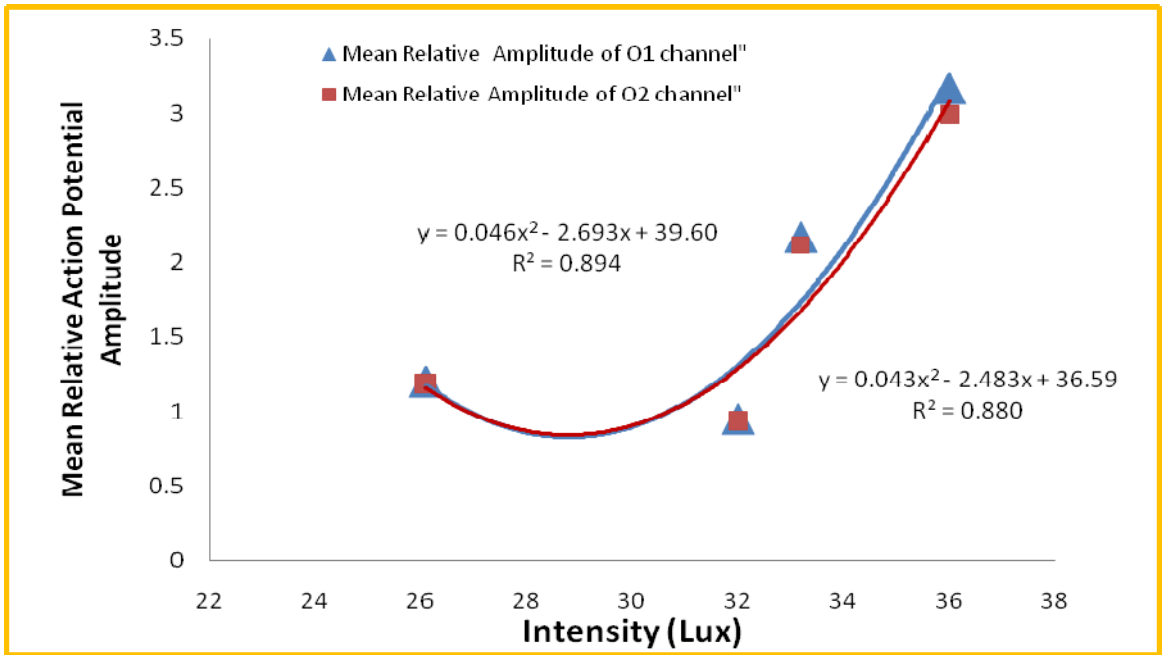

Figure (2): The relation between the intensity of yellow color (Lux) and the mean relative action potential amplitudes for females of O_1 and O_2 channels

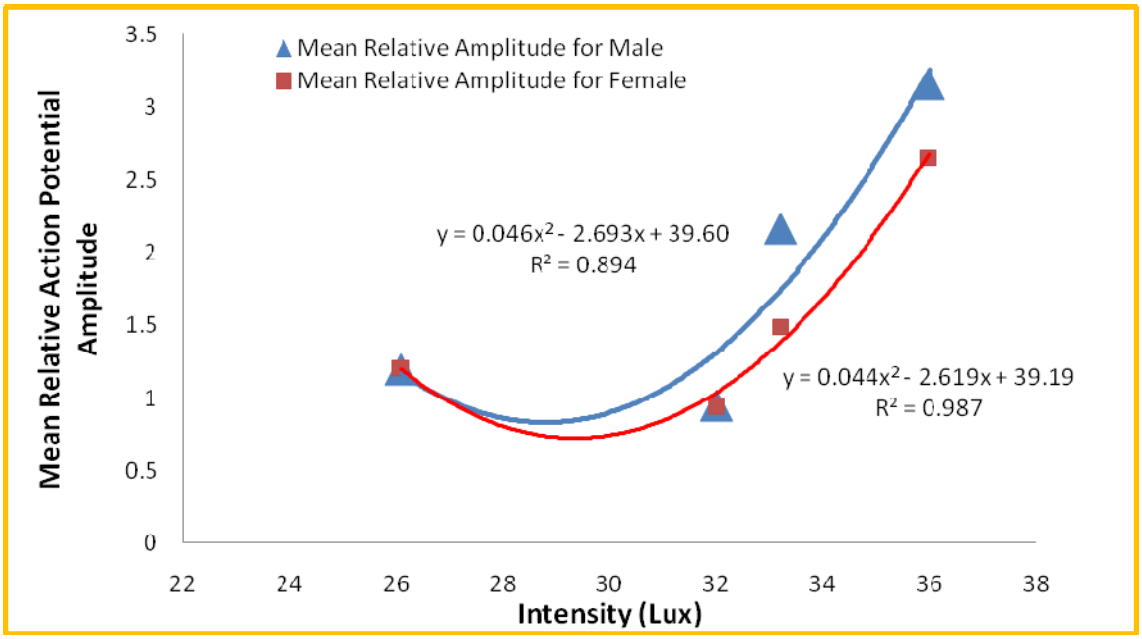

Figure (3): The relation between the intensity of yellow color (Lux) and mean relative action potential amplitudes for males and females of O_1 channel.

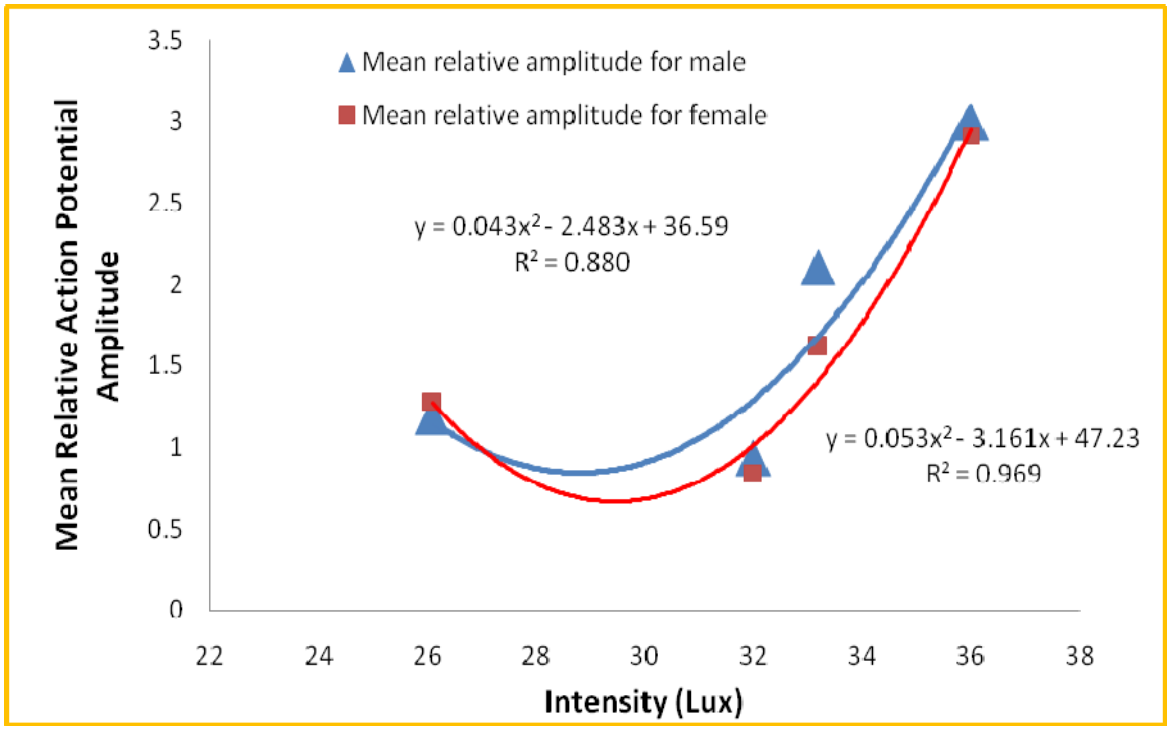

Figure (4): The relation between the intensity of yellow color (Lux) and mean relative action potential amplitudes for males and females of O_2 channel.

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هەلسەنگاندنا نەخشە شەپولێن ئەلکتریکی یێن مەژی ل جهێ هەستیار بو بیناهیێ بکارئینانا دریژیا شەپولێت فوتونا زەر بو چرییێت جودا جودا

پوختە

کارەبا هێلەشیکاریا مەژی جورە پیڤانەکە بو فەسلەجا دەمارا بکارئینانا جەمسەرێت دئێنە گرێدان ل سەر کلوخێ سەری یان ژی هندەك جارا ل سەر پیستێ سەری.

ئەنجامێت هیلەکاریێ ئەوێت ئێنە ناسکرن ب (EEG)یان ژی دبێژنێ شەپولێت مەژی. کارەبا هێلەشیکاریا مەژی دئێتە بکارئینان بو وان نەخوشا ئەوێت نەخوشییێت مەژی هەین کو ئاریشە هەین د ساخلەتێن خو یێن دەرونی وەکی نەشیانا وان بو فێربینێ یان ژی دان و ستاندنەکا نورمال د ناڤ جڤاکیدا دبنە بارگرانیەك ل سەر جڤاکی .

ئەڤ ڤەکولینە یێ هاتیە کرن بو تومارکرنا شەپولێت EEGبو کەنالێت (O_1, O_2) ئەوێت گەلەك هەستیار بو دریژیا جودابیێت شەپولێت فوتونا زەر بو چرییێت جودا جودا وتێنە بەراوردکرن ل گەل شەپولێت EEG بو هەردوو توخمێ نێرو میێا بو گروپێت ژیێ وا نێزیکی ئێك .

سامپلێت دەست نیشانکرن بو ڤێ ڤەکولینێ پێك دێن ژنەهـ نێر و وەکی وان مێ ئەوێت خو بەخش ژیێ وان دناڤبەرا ٢٥–٢٠ سال هاتنە دەست نیشانکرن بو تاقیکرن سەر بێتە کرن .

دئەنجامدا کارتێکرنا ماتە ووزێ ئەوا هاتیە تومارکرن ژ کەنالێ O_2 , بو توخمێ نێر وەك ئێکە د چرییێن نزم دا ودئێتە زیدەکرن دگەل زێدەکرنا چریێ ژ کەنالێ O_1 و هەر وەسا بو بو توخمێ مێ.

بەراورد کرن د ناڤبەرا نێر و مێ دا خویا کر کو توخمێ نێر گەلەك هەستیارترن بو رەنگێ زەر ژ توخمێ مێ بو هەمی تاقیکرنێ روناهیێ د هەردوو کەنالا دا .

تقييم تخطيط الاشارات الدماغية في منطقة الرؤيا المحفزة باستخدام فوتونات اللون الاصفر معين الطول الموجي وبكثافات مختلفة

الملخص:

يعتبر تخطيط الدماغ كهربائياً مقياساً لتغير النشاط الكهربائي الفسلجي العصبي المستثار طبيعياً (ارادي او لا ارادي) او متاثراً بمحفز خارجي والمسجل على شكل موجات كهربائية باستخدام اقطاب معدنية تربط على فروة الرأس أو في حالات خاصة على اللحاء وهذه الموجات تختلف ترددتها باختلاف قوة المحفز ونوعيته فيزيائياً كان , كيميائياً , أو ميكانيكي والتي تظهر الاستجابة لها في المناطق ذات العلاقة بالتحسس على شكل فرق جهد كهربائي.

هذا البحث يهتم بالمحفز الفيزيائي الضوئي للون الاصفر احادي التردد (515 THz ≈)وتسجيل مدى الاستجابة له من قبل المستلم في الشبكية ونقله عبر العصب البصري ومن ثم الاستجابة من قبل منطقة الرؤيا في الدماغ التي تسجل بواسطة (EEG).

عينة البحث مكونة من (١٨) متبرع سليمي حاسة الرؤيا مقسمين الى تسع ذكور وتسعة اناث وأعمارهم تتراوح بين ٢٠-٢٥ سنة , كل احتمالات تداخل الموجات الكهربائية المتداخلة مع الاستجابة للمؤثر سواءً كانت تداخلات فسلجية ارادية او لا ارادية أو بتأثير اشارات كهربائية في المحيط الخارجي او المنظومة الكهربائية للفحص قد اخذت الاحتياطات لحذفها او لإيصالها لمستويات الاهمال.

اوضحت النتائج بان معدل الازاحة لفرق الجهد الكهربائي النسيي الفاعل لقنوات تسجيل محفز الرؤيا (لفوتونات اللون الاصفر) المسجلة في القناة (O_2) لمجموعتي الذكور والإناث متساوية عندما تكون الشدة قليلة ومن ثم تزداد مع ازدياد الكثافة الضوئية للفوتونات مما هو مسجل في القناة (O_1) وان معادلة العلاقة بين المعدل النسيي لإزاحة فرق الجهد (Y) مقابل الكثافة الضوئية (x) للقناتين اعلاه للذكور وعلى التوالي هي $y = 0.053x^2-3.161x + 47.23$ مع $R^2 = 0.969$ و $y = 0.044x^2- 2.619x + 39.19$ مع $R^2 = 0.987$ وللاناث هي $y = 0.046x^2 – 2.693x + 39.60$ مع $R^2 = 0.894$ و $y = 0.043x^2- 2.483x + 36.59$ مع $R^2 = 0.880$.

وللمقارنة بين الاشارات الكهربائية المسجلة من القنوات (O_1 & O_2) الذكور والإناث فقد وجدنا بان المعدل النسيي للإزاحة لفرق الجهد الفاعل ولكلا القناتين هو اعلى في الذكور منه في الاناث ولكافة الكثافات الضوئية للون الاصفر المستعمل في البحث وهذا يدل بوضوح بان مناطق الرؤيا عند الذكور هي اكثر تحسساً لهذا اللون من الفوتونات مما هو في الإناث. معادلات العلاقات النموذجية للذكور ولكلا القناتين على التوالي كانت $y =0.046x^2 – 2.693x + 39.60$ مع $R^2 = 0.894$, $y = 0.044x^2 – 2.619x + 39.19$ مع $R^2 = 0.880$, بالنسبة للإناث وعلى التوالي هي $y =0.043x^2 - 2.483x + 36.59$ مع $R^2 = 0.880$, $y = 0.05x^2 – 3.61x +$ 47.23 مع $R^2 = 0.969$.

گۆڤارا زانکۆیا زاخۆ

أ - زانست

پەربەندا (المجلد) ٢
هژمارا (العدد) ١

خزیران
حزیران
٢٠١٤

هەرێما کوردستانا عیراقێ
وەزارەتا خویندنا بالا
وتوێژینەوەی زانستی
زانکــۆیــا زاخـــۆ

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