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FORWARD

It is my pleasure to welcome you back and present you this issue, Volume 5 , No. 1 (2010), of ***International Journal for Sciences and Technology (IJST)***. The members of Editorial Board, the ICAST and TSTC team work and I hope you will find this collection of research articles useful and informative.

The journal is one of the scientific contributions offered by ***the International Centre for Advancement of Sciences and Technology*** to the science and technology community (Iraqi, Arab Region and International).

Finally, on behalf of *the International centre*, I would like to express my gratitude and appreciation to the efforts of the Editorial Board, Researchers and ICAST and TSTC Team Works for managing the scientific, design, technical and administration aspects of the Journal and for preparing this volume for final printing and publishing.

Editor-in-Chief
IJST
Abdul Jabbar Al- Shammari

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Relationship between blood group and *H.pyloria* infection in Jordan

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ABSTRACT

Helicobacter pylori infection occurs worldwide, but the prevalence varies greatly among countries and among population groups studied. People with blood group O have been noted to be more susceptible to H. pylori infection.

In Jordan there is no such study have been conducted. This study aimed to determine the relationship between the ABO blood groups and H.pylori infection in adult's patients with and without dyspeptic symptoms attending Al-Zarka General hospital and Internal medicine private clinic inside Amman.

Out of 197 subject, 121 (65%) were males and 76 (36%) females with mean range age of 32.7 years. Immunological analysis reveals that 112 (56.8%) and 85 (43.2%) were found positive and negative for H.pylori infection respectively.

Group O was the most prevalent blood group (54.3%) followed by A (24.5 %), B (16.7%) and AB (4.5 %). Further analysis on the ABO blood groupings and seroprevalence of H. pylori demonstrated that seropositive rate of H. pylori was 66.7% in blood group O, 52.2% in blood group A, 43.8% in blood group B and zero% in blood group AB. Statistical significant between subject in blood group AB and other groups.

This study concludes that the seroprevalence of H.pylori infection in Jordan was 56.8%. There was no statistically significant association between H. pylori

infection and sex and ABO blood groups.

Keywords: *H.pylori* infection, ABO/blood group

INTRODUCTION

The associations of ABO blood groups and various disease were studied before (1, 2, & 3), and the ensuing vitriolic debates. Helicobacter pylori infection is the most common chronic bacterial infection in the world.

Seroepidemiologic studies indicated that about 50% of adults in the developed countries and nearly 90% of adults in developing countries were seropositive for H.pylori (4). Chronic *H. pylori* infection may be associated with chronic gastritis, peptic ulcer disease [(5)], mucosal associated lymphoid tissue (MALT) lymphoma and gastric adenocarcinoma [(6)].

Although a number of epidemiological studies have suggested a higher prevalence of *H. pylori* infection in patients with dyspepsia, only few high quality therapeutic trials have specifically investigated whether *H. pylori* infection causes dyspepsia[(7)].

Blood group specificities of the ABO system are genetically determined, stable host characteristics which have been associated with susceptibility to some infectious diseases. The prevalence of blood group O is increased in patients with typhoid ,paratyphoid and cholera; group B is increased in patients with gonorrhea, Chlamydia infection, urinary tract infection caused by *E.coli* and in the children with gram negative enteric infection; and there are reports of an increased prevalence of group A among patients with meningococcal meningitis (8,9,10).A second genetically controlled host characteristic which has been associated with the susceptibility to infection in the inability to secret the water soluble glycoprotein forms of ABO blood group antigens (11).

Linden et al., has a novel observations suggest that the individual ABO blood group and secretor phenotype are part of human and non-human primate innate immunity against infectious disease (12). These phenotypes

are associated with the susceptibility to infection in the inability to secret the water soluble glycoprotein forms of ABO blood group antigens (13). They related the ABO antigens in to three phenotypes; secretors (representative by AB blood group antigens), weak-secretors (group A & B) and non-secretors (group O) (14, 15).

The prevalence patients of non-secretors is increased among patients with cholera (16), urinary tract infection caused by *E.coli* (8), candidasis (17), meningococcal meningitis and pneumococcal infections (10), but not among those with tuberculosis, leprosy and gonorrhea(18). Focus to association between ABO blood group and H.pylori infection, Tadege et al., reported no statistical significant association between H.pylori infection and ABO blood group in Ethiopian patients (19). Several studies in Iran (20,21) ,Turkey (22,23) and Greece (24), reported the same finding. In contrast, Kanbay et al. observe red that individuals with blood group A & O were more prone to *H.pylori* infections, and those of AB blood group were less prone (25).

This study aimed to determine the relationship between the ABO blood groups and *H.pylori* infection in adult's patients with and without dyspeptic symptoms in Jordan.

MATERIALS AND METHODS

Subject of study: One hundred and ninety seven individual, 110 were suffering from dyspepsia , peptic and gastric ulcer included 65 adults male and 45 femal, while the other 86 health individuals does not suffered from these symptoms; 55 adult males and the other were female. The patients were visit the Al-Zarka general hospital and private clinic in Amman, Jordan.

Samples collection: Five ml venous blood was collected from each informed and consenting adult dyspeptic patients and non-dyspeptic (healthy individual). ABO/Rh blood grouping were performed using commercial kit (Arab diagnostic Kit Company, Amman, Jordan). The sera were obtained from blood after

centrifugation (300 rpm for 10 minutes).

Detection of *H.pylori*: All the 197 sera collected from patients were examined for the presences of antibodies against *H.pylori. H.pylori* kit (ACON Laboratories, Inc), a one step test device is rapid chromatographic immunoassay for qualitative detection of antibodies to *H.pylori* in serum (26). Briefly, the test device was placed on the clean and level surface. Transfer 3 drops of serum (approx. 100μl) to the specimen well (s) of test device.

Test was observed after 10 minutes. Results interpretation done by formation of two distinct red lines, one line representative the control region and the other line representative the test region. In case of negative test, only one red line appeared in the control region, no apparent red or pink line appeared in the test region. Low levels *H.pylori* antibodies result in a faint line appeared in the test region, we extended time and read it after 30 minutes.

Statistical analysis: Ready programmed EPI-INFO version 2000 (CDC, Atlanta, Georgia, USA) with Yates's correction and pearson Chi-square were applied and P values ^ 0.05 were considered as statistically significant.

RESULTS

The most prevalent blood group in Jordanian individual tested was type O (54.3%) followed by A (24.5 %), B (16.7%) and AB (4.5 %) as shown in Table1. Further analysis on the ABO blood groupings and seroprevalence of *H. pylori* demonstrated that seropositive rate of *H. pylori* was 66.7% in blood group O, 52.2% in blood group A, 43.8% in blood group B and zero% in blood group AB (Tables 1 & 2). Rh positively was also higher in patients than in controls (*P* ~ 0. 05) (Table 1). *H. pylori* positivity was similar between blood groups among patients.

Table1. Relationship between ABO blood groups and seropositivity to H.pylori infection.

Blood group	Total tested	Sub blood group	Results	Positive *H.pylori*	Negative *H.pylori*
O	107	O+	90	61	29
		O-	17	11	6
A	48	A+	40	21	19
		A-	8	4	4
B	33	B+	27	13	14
		B-	6	2	4
AB	9	AB+	9	Zero	9
		AB-	Zero	Zero	Zero
Total	**197**	**----------**	**197**	**112**	**85**

Table 2. Percentage of individuals subjected to study

Blood group	Total tested No. (%)	HP Positive No. (%)	HP Negative No. (%)
O	107(54.3%)	72(66.7%)	35(33.3%)
A	48(24.5 %)	25 (52.2%)	23(47.8%)
B	33(16.7%)	15(43.8%)	18(56.2%)
AB	9(4.5 %)	Zero (0%)	9(100%)
Total	**197(100%)**	**112(56.8 %)**	**85(43.2 %)**

There was statistically significant difference in seroprevalence of *H.pylori* infection between healthy and dyspeptic patients (P`0.05) (data not shown). On other hands, there was statistically significant difference in *H.pylori* seropositivity between male and females (table 3). This study reveals that the incidence of *H.pylori* infection in male (64.2%) superior than female (35.8%). No statistical significant association between individuals in ABO blood group A, B, and O with *H.pylori* infection in both male and female (table 3).

Table3. Relationship of ABO blood groups and H. pylori infection dependent on sex.

Blood group	Male (121)		Female (76)	
	Positive H.pylori	**negative H.pylori**	**Positive H.pylori**	**negative H.pylori**
O	46	23	25	13
A	16	12	9	11
B	9	8	6	10
AB	Zero	7	Zero	2
Total	**71 (64.2%)**	**50 (58.5%)**	**40 (35.8%)**	**36 (41.5%)**
	121 (61.7%)		**76 (38.3%)**	

DISCUSSION

Since 1950's and 1960's, several reports mention that individuals of blood group O and these who are non-secretors of the glycoprotein form of their ABO blood group antigens are over-represented among patients with gastric or duodenal ulcer (18,27,28).

In our study, we found that there is no statistical significant association between ABO blood group and seroprevelance of *H.pylori*. H*.pylori* positivity was similar between blood group among individuals in study. Blood group AB was differs and shows high resistant to *H.pylori* infection. Most studies have revealed no association between the ABO blood groups and *H.pylori* infections, either in healthy (23, 29), or in symptomatic subjects (30, 31, 32.). Though only few studies have been reported, a pattern is emerging of an association between non-secretion of blood group antigens and susceptibility to infections of mucosal surface (33, 34, 35, 36).

Several reports discussed the relationship between the secretors and non-secretors glycoprotein from ABO groups (8, 11, 37, 38), and still the biological function of the ABO blood group antigens has remained an enigma. Linden et al. shows that the great majority of Rhesus monkeys are of blood

group B and weak-secretors (12). This observation suggests that an evolutionary adaptation in digestive tract mucosal carbohydrate patterns to local environmental selection has occurred. Also they demonstrate that long-term infection by the "peptic ulcer bacterium" *Helicobacter pylori* induces mucosal carbohydrate patterns that change according to the individual secretor phenotype (13, 14). The common weak-secretor monkeys were apparently "protected," as they had stable glycosylation, lower inflammation, and lower bacterial infection load, whereas the less common secretor animals had increased levels of inflammation-associated mucosal carbohydrate patterns and a transient decrease in the ABO blood group system type of carbohydrates (38, 39). These novel observations suggest that the individual ABO blood group and secretor phenotype are part of human and non-human primate innate immunity against infectious disease (12, 40, 41, and 42). It seems that reasonable explanations to the relationships between ABO blood group and diseases in general, and *H.pylori* infection in special concern. Other facts that the races play a significant role in this association. For example, since the majority of Caucasians (80%) are secretors, whereas 20% of them are non-secretors and weak-secretor individuals are rare or not yet discovered. In contrast, weak-secretor individuals are common among Chinese, Japanese, Polynesians, Australian aborigines, and African-Americans (37). These displinces between races brought our attention to conduct this study for Jordanian individuals, even though we need further studies to approach such concussion. It seems that the Mediterranean and south west Asia are alike for susceptibility to *H.pylori* infections (20, 21, 22, 23, 24, 25). The skewed prevalence in secretor phenotypes suggests selection in response to specific types of infections or other environmental conditions.

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Enhancement of secondary metabolites production in *in- vitro* cultures of *Salvia officinalis*

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ABSTRACT

In an attempt to increase the production of some secondary metabolites in tissue cultures of Salvia officinalis in comparison with the intact plant, several experiments were carried out. Callus was induced and maintained on MS medium supplemented with 0.5mg/l kinetin and 0.05mg/l 2,4-D from leaf and stem explants. NaCl was added to the culture medium at concentrations (50 or 100)mM as a stress agent for elicitation.

Gas chromatography technique was used to identify and quantify the compounds. Results showed that α-pinene increased more than four folds in callus cultures initiated from leaf and grown on a medium containing 100mM NaCl compared with the same explant excised from the intact plant. The above mentioned medium also increased apigenin and linalool production more than three folds. Rutin increased up to 2.5 times in cell suspension cultures initiated form stem explants. Other compounds such as geraniol, quercetin and coumarin increased at different ratios using tissue culture systems. Alkaloids and steroids were not detected neither in intact plant nor tissue cultures.

Keywords: *Salvia officinallis*, secondary metabolites, tissue cultures, elicitors.

INTRODUCTION

Plants have been an important source of medicine for thousands of years and are also the source of many modern medicines (32). It is estimated that approximately one quarter of prescribed drugs contain

plant extracts or active ingredients obtained from plant substances (16). The most popular analgesic, aspirin and some of the most valuable anti-cancer agents such as paclitaxel and vinblastine are derived solely from plant sources (35).

Garden sage, *Salvia officinalis*, is a medicinal herb that has long been used in popular medicine (34). The majority of sage is related to its content of an important active constituents mainly volatile oils, that composed of monoterpenes (e.g. linalool, geraniol, pinen and thujone) and sesquiterpenes, phenolic compounds as phenylpropanoids (e.g. coumarins), flavonols and flavons (e.g. quercetin, apigenin, rutin and luteolin), glycosides, tannins, and many other important compounds that have value as pharmaceuticals (36; 19).

Gas liquid chromotography methods are used for separation, identification and quantification of the extracted compounds (5).

The production of secondary metabolites *in vitro* is possible through plant tissue culture (4; 8). *In vitro* study holds a potential for the production of high-quality plant based medicines (21). This can be achieved through different methods including micropropagation of cell lines capable of producing high yield of secondary compounds in cell suspension cultures (37). The accumulation of secondary products in plant cell cultures depends on many factors including the composition of the culture medium and environmental conditions (29). The addition of stress agents to the culture medium may increase the formation of secondary metabolites in cultured cells (26).

As a result of the importance of this locally grown plant as a potential source of phytochemicals, this research work was aiming to: Detection and quantification of some essential oil compounds (α-pinene, geraniol and linalool) and phenolic compounds (apigenin, rutin, coumarins and quercetin) which have a medicinal value in intact plant using GC technique, initiation of tissue cultures from the plant, then examination of the cultures for the existence of these metabolites, inclusion of NaCl to culture medium as a stress

stimulus agent in an attempt to increase the production of such secondary metabolites and comparison between productivity of such compounds between intact plant parts and tissue cultures after and before NaCl addition.

MATERIALS AND METHODS

Sage plants, *Salvia officinalis* (Labiatae) were purchased from local nurseries in 12cm clay pots. MS (20) medium was prepared and used. Leaf disks and stem explants were surface sterilized using sodium hypochlorite at a concentration of 3% for 3min., then rinsed with sterilized distilled water for 3 times. Sucrose 30000mg/l, myoinositol 100mg/l and the plant growth regulators (2,4-D and kinetin) at different concentrations were added. The pH was adjusted to 5.8, then 7g/l of the agar type (Agar-Agar) was added to the medium, placed on a hot plate magnetic stirrer till boiling, then aliquots of 10ml were dispensed into (8 ×2.5) cm culture vessels. The medium was left at room temperature to cool and become ready to culture explants. Culture media were sterilized by autoclaving at 121°C under (1.04Kg/cm^2) pressure, for 15min. while glassware and other instruments either by autoclaving or using electric oven (180-200) °C for 2hrs (6).

Surface sterilized explants 1cm long were inoculated into the culture vessels under aseptic conditions, placed in the incubator (Sanyo Electric Co., Ltd.) at 25°C for 16/8hrs light/dark photoperiod using day light inflorescent and light intensity of 1000lux was used.

Different combinations of 2,4-D and kinetin were examined to determine the most effective one for callus induction. Cultures were placed in the incubator. The response of these explants was evaluated after 21day in culture to determine the proper combination for callus induction.

The initiated callus was removed from the explants using forceps and scalpel, then pieces weighting 50mg were subcultured onto fresh medium supplemented with the same combinations of 2,4-D and kinetin. Callus fresh weight was determined using sensitive balance, and then oven dried at

40°C for 24hrs (3) for callus dry weight measurements and for extraction to be used in Gas Chromatography (GC) work .

Cell suspension cultures were initiated by placing 5g callus pieces from stem or leaf explants origin into 100ml of maintenance medium in 250cm³ flasks which placed on a rotary shaker at 100rpm/min for 21day.

Suspension cultures were filtered and the pellet was oven dried at 40°C for 24hrs to be ready for use in GC work.

Ten callus pieces (400mg) each were placed on the surface of callus maintenance medium supplemented with (50 or 100) mM NaCl for 21day, then dried at 40°C for 24hrs. for extraction and analysis of the of secondary metabolites.

Samples harvested from intact plant taken from stems and leaves, from callus and cell suspension cultures initiated from both stems and leaves, placed on a medium supplemented with or without elicitor (NaCl 50 or 100)mM. All these samples were oven dried at 40°C for 24hrs. (3), ground into a powder using a pestle and mortar, then subjected to ethanolic extraction.

Gas chromatography method was used for identification of compounds. The analysis was performed using GC-9A Shimadzu column supele Co wax 10 (15ft × 1/8 1n) stainless steel, internal diameter 2.1mm, oven temperature was programmed on 50°C for 2min., then increased to 200°C at a rate of 2°C/min., helium flow rate 50cm/sec., with flame ionization detector as specified by the manufacturer.

Standards of terpenes and flavonoides were obtained from Aldrich Co. U.S.A., as pure standards. A sample of 0.1mg of each dry weight was mixed thoroughly with 0.5ml ethanol. The supernatant was separated and 10µl was injected into the GC column and the retention time was determined. The area for each peak was calculated and compared with the known concentration of the prepared samples (13). The concentration of each compound was calculated using the equation (14):

$$\text{Conc. (mg/g)} = \frac{\text{area of sample}}{\text{area of standard}} \times \text{conc. of standard}$$

A completely randomized design (CRD) was used with 12 replicates. Least significant differences (LSD) were obtained to compare means at probability of ≤0.05. For secondary metabolite quantification, means were calculated and standard errors were computed for three sample replicates (12).

RESULTS AND DISCUSSION

The effect of different concentrations of kin and 2,4-D on the percentage of leaf and stem explants responded to callus induction is shown in pictures (1 and 2) respectively.

Picture 1: Callus induction on leaf explants grown on MS medium containing a combination of 0.5mg/l kin and 0.05mg/l 2,4-D, 21day after culture.

Picture 2: Callus induction on stem explants grown on MS medium containing a combination of 0.5 mg/l kin and 0.05mg/l 2,4-D, 21day after culture.

There was a significant increase in the (%) response with increasing kin concentrations up to 0.5mg/l. Maximum response reached 41.68% with explants treated with 0.5mg/l, which not significantly different from the level 1.0mg/l. Minimum response percentage induction was recorded in a medium deficient of kin (13.32 %).

Callus cultures induced on leaf explants from the best combination of kin and 2,4-D (0.5 and 0.05)mg/l respectively, were inoculated into the same combinations of plant growth regulators used for callus induction to determine the appropriate concentration for callus maintenance (Table 1).

Inclusion of kin at the concentration of 0.5mg/l gave significantly higher callus fresh weight (324.58mg) than other concentrations, while the lowest was in the treatment where no kin was added to the culture medium. The highest callus fresh weight obtained in 2,4-D treated callus cultures (464.84mg) was at the concentration 0.05mg/l (Picture 3). This fresh weight was significantly higher than other treatments.

Table (1): Effect of different concentrations of 2,4-D and kinetin on callus fresh weight (mg) initiated on leaf explants of S. officinalis grown on a maintenance medium. Initial weight was 50mg. (n= 12).

2,4-D (mg/l)	Kinetin (mg/l)				Mean
	0.0	0.1	0.5	1.0	
0.0	0.00	514.62	335.83	234.17	271.16
0.05	151.19	171.25	915.07	621.85	464.84
0.1	132.70	180.15	117.50	245.22	168.89
0.5	115.13	160.02	148.57	301.20	181.23
1.0	103.95	112.22	105.93	0.00	80.53
Mean	100.59	227.65	324.58	280.49	
LSD 0.05	kin =40.6 2,4-D= 36.8 kin× 2,4-D= 74.7				

The interaction between the two growth regulators resulted in maximum callus production reached (915.07mg) at the levels of (0.5 and 0.05)mg/l kin and 2,4-D respectively. This was significantly higher than all other interactions. Callus tissues showed a reduced growth when inoculated into media lacking or containing 1.0mg/l kin and 1.0mg/l 2,4-D.

Picture 3: Callus cultures originated from leaf explants grown on a maintenance medium containing0.5mg/l kin and 0.05mg/l 2,4-D. Cultures were continuously cultured on fresh medium at 21day intervals.

Table (2) indicates that the trend was similar in callus cultures initiated on stem explants since the highest fresh weight (836.67mg) was obtained from the combination of 0.5mg/l kin and 0.05mg/l 2,4-D (Picture 4). This fresh weight was significantly higher than other combinations.

Table (2): Effect of different concentrations of 2,4-D and kinetin on callus fresh weight (mg) initiated on stem explants of S. officinalis grown on maintenance medium. Initial weight was 50mg. (n = 12)

2,4-D (mg/l)	Kinetin (mg/l)				Mean
	0.0	0.1	0.5	1.0	
0.0	0.00	392.33	218.28	180.48	197.77
0.05	123.08	114.48	836.67	552.05	406.57
0.1	133.20	124.25	90.32	209.87	139.41
0.5	97.18	118.77	130.72	199.65	136.58
1.0	96.43	102.17	0.00	0.00	49.649
Mean	89.98	170.40	255.20	228.41	
LSD 0.05	kin= 37.2 2,4-D= 42.6 kin× 2,4-D= 78.7				

Picture 4: Callus cultures originated from stem explants grown on maintenance medium containing 0.5mg/l kin and 0.05mg/l 2,4-D. Cultures were continuously cultured on fresh medium at 21day intervals.

Dry weights of callus cultures initiated from both leaf and stem explants are shown in tables 3 and 4 respectively.

Table (3): Effect of different concentrations of 2,4-D and kinetin on callus dry weight (mg) initiated on leaf explants of *S. officinalis* and grown on maintenance medium (n= 12).

2,4-D (mg/l)	Kinetin (mg/l)				Mean
	0.0	0.1	0.5	1.0	
0.0	0.00	82.86	71.09	28.72	45.66
0.05	53.63	21.00	107.95	92.80	68.85
0.1	16.63	24.59	15.33	35.88	23.11
0.5	12.80	25.96	23.83	39.63	25.56
1.0	10.64	15.25	13.97	0.00	9.97
Mean	18.74	33.93	46.43	39.41	
LSD 0.05	kin= 6.3 2,4-D= 6.8 kin× 2,4-D= 10.6				

Table (4): Effect of different concentrations of 2,4-D and kinetin on callus dry weight (mg) initiated on stem explants of *S. officinalis* and grown on maintenance medium (n= 12).

2,4-D (mg/l)	Kinetin (mg/l)				Mean
	0.0	0.1	0.5	1.0	
0.0	0.00	49.33	32.58	24.22	26.53
0.05	15.28	17.92	115.67	73.61	55.62
0.1	18.17	17.97	14.91	30.90	20.49
0.5	14.11	17.14	18.90	25.61	18.94
1.0	12.54	13.36	0.00	0.00	6.48
Mean	12.02	23.14	36.41	30.87	
LSD 0.05	kin= 5.4 2,4-D= 8.3 kin× 2,4-D= 12.3				

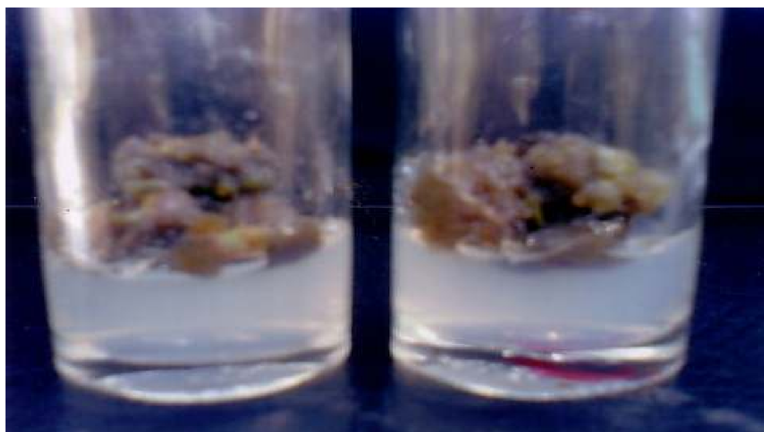
The combination of 0.5mg/l kin and 0.05mg/l 2,4-D showed the highest dry weights in both types of explants. These weights (107.95mg) and (115.67mg) were significantly higher than all other treatments. According to the results stated above, callus was induced on leaf and stem explants then maintained for many subcultures on MS medium containing 0.5mg/l kin and 0.05mg/l 2,4-D for subsequent experiments. Induction and maintenance of callus cultures in *S. officinalis* seem to favor low levels of 2,4-D and rather higher levels of kin Increasing the levels of the two plant growth regulators suppressed callus growth. The increase of callus mass is important for the production of secondary metabolites since they are proportionally related (25). It would be convenient from the practical point of view to induce and maintain callus on the same growth nutrients and plant growth regulators requirements. Induction of callus on both types of explants using the same medium components is an additional advantage for plant biotechnologists.

The addition of 100mM NaCl to MS medium caused browning to callus culture, while callus weight was not much affected for both concentrations (50 and 100)mM of NaCl. The callus browning may relate to the increase in secondary products secretion especially the phenolic compounds. Callus growth

initiated from leaf explants and grown on the maintenance medium containing 100mM NaCl was more clumpy, unfriable and tended to be yellowish (Picture 5). While callus cultures initiated from stem explants tended to be friable and brown (Picture 6).

GC methods were used for detection and quantification analysis of (rutin, α-pinene, linalool, geraniol, apigenin, quercetin and coumarin). Quantities of the investigated secondary metabolites are presented in table (5). The quantities varied depending on the type of plant tissue and the type of culture. Rutin showed the highest concentration in callus culture that initiated from leaf and grown on a medium supplemented with 100mM NaCl as elicitor reaching (0.448mg/g) followed by cell suspension culture initiated from stem explants (0.418mg/g), while the lowest value was in stem explants of the intact plant and in the sample of callus culture initiated from stem explants an grown on a medium supplemented with 50mM NaCl (0.162 and 0.166)mg/g respectively. α-pinene showed the highest concentrations (1.390mg/g) in callus cultures initiated from leaf and grown on a medium supplemented with 100mM NaCl. The lowest concentrations of α-pinene

Picture 5: Appearance of callus cultures initiated originally on leaf explants and maintained on maintenance medium containing 100mM NaCl as elicitor.



Picture 6: Appearance of callus cultures initiated originally on stem explants and maintained on maintenance medium containing 100mM NaCl as elicitor.

were shown in samples of cell suspension culture initiated from stem (0.141mg/g) followed by the samples of intact plant from leaf source (0.298mg/g). α-pinene was not detected in callus cultures that initiated from leaf and stem explants and grown on a medium supplemented with 50mM NaCl and in the sample of callus culture initiated from stem and grown on a medium supplemented with 100mM NaCl as elicitor. Maximum concentration of linalool appeared in callus cultures initiated from leaf and stem explants grown on a medium supplemented with 100mM NaCl (1.170 and 1.137)mg/g respectively. The lowest concentrations were found in callus samples initiated from leaf and grown on a medium supplemented with 50mM NaCl (0.230mg/g) followed by leaf explants of the intact plant with a value of (0.330mg/g). Linalool was not detected in callus cultures initiated from stem explants. Geraniol was detected in all samples achieving the highest level in callus cultures initiated from leaf and grown on a medium supplemented with 100mM NaCl (0.547mg/g) and in cell suspension cultures initiated from leaf explants (0.524mg/g). The lowest concentration was detected in callus cultures initiated from stem and grown on a medium supplemented with 100mM NaCl (0.076mg/g) and in callus cultures initiated from stem (0.093mg/g). Apigenin was found at high concentration in callus cultures initiated from leaf and grown on a medium supplemented with 100mM NaCl (0.825mg/g) followed by (0.758mg/g) in cell suspension

cultures of stem explants. Callus cultures initiated from stem explants recorded the lowest concentration (0.050mg/g) also the sample of the intact plant from stem recorded low apigenin content (0.098mg/g). Quercetin recorded the maximum concentration in the sample of callus cultures initiated from leaf explants and grown on a medium supplemented with 100mM NaCl(0.768mg/g), whereas the lowest level was detected in callus cultures initiated from stems and grown on a on a medium supplemented with 50mM NaCl. Quercetin was not detected in cell suspension cultures initiated from leaf explants. Coumarin highest level (0.590mg/g) was found in callus cultures initiated from leaf and grown on a medium supplemented with 100mM NaCl followed by (0.440mg/g) in callus cultures initiated from stem and grown on a medium supplemented with 100mM NaCl. Coumarin was not detected in cell suspension cultures initiated from stem explants.

It is clear that α-pinene was abundant at high concentration compared with other studied compounds. The table also shows that among all the samples under investigation, the callus culture initiated from leaf and grown on a medium supplemented with 100mM NaCl as elicitor gave the highest concentrations for all tested essential oils and phenolic compounds.

Fried and Sherma (11) and Culea *et al*., (7) reported that the separation and purification of secondary products are usually carried out using gas chromatography and TLC methods.

Exposure of cultured plant cells to an elicitor result in some genes expression that leads to the formation of the secondary metabolites which are found in entire plant. Additionally, inclusion of NaCl in culture medium may induce the synthesis of new proteins (2). This may lead to the synthesis of secondary metabolites that plant tissue cultures are utilized for stress tolerance. Although the undifferentiated cells of plant tissue cultures are generally totipotent, many genes including those involved in secondary metabolism are repressed with the consequence that the yields of desired compounds in cultures are low.

However, it is becoming increasingly apparent that a large

number of secondary metabolites belong to a class of substances termed phytoalexins. These are stress- related compounds produced in the normal plant as a result of damaging stimuli from physical, chemical or microbiological factors. When cell cultures are subjected to such elicitors, some genes are depressed, resulting in the formation of the secondary metabolites which are found in the entire plant.

The results agree with Taniguchi (31) who stated that terpenoid synthesis can be induced by elicitors and Phatak (24) who reported that the hydrocarbons (terpenes) can be detected in tissue cultures. Wide variety of chemicals can be produced from plant tissue cultures, some produce medicines, essential oils and various other biochemicals.

Stojakowska (30) reported that various types of phenolics and polyphenols are constituents of plant tissue cultures.

Vanisree *et al.,* (33) referred to the major advantages of a cell culture system over the conventional cultivation of whole plants which are: Useful compounds can be produced under controlled conditions independent of climatic changes or soil conditions, cultured cells would be free of microbes and insects, cells of, tropical or alpine, plants could easily be multiplied to yield specific metabolites, automated control of cell growth and rational regulation of metabolite processes would reduce labor cost and improve productivity and organic substances are extractable from callus cultures easily.

Table (5): Quantification of secondary metabolites (mg/g) detected in intact plant and different cultures initiated in vitro

Source	Explant	Rutin	α-pinene	Linalool	Geraniol	Apigenin	Quercetin	Coumarin
Intact plant	Leaf	0.338	0.298	0.329	0.309	0.225	0.420	0.411
		0.019	±0.009	±0.018	±0.007	±0.013	±0.006	±0.009
	Stem	0.162	1.347	0.755	0.153	0.098	0.138	0.298
		±0.017	±0.067	±0.008	±0.016	±0.001	±0.002	±0.022
Callus culture	Leaf	0.410	0.307	0.906	0.110	0.146	0.269	0.313
		±0.024	0.032	±0.003	±0.003	±0.002	±0.131	±0.066
	Stem	0.357	0.330	0.000	0.093	0.050	0.163	0.33
		0.049±	±0.052	0.000	0.020±	0.001±	0.015±	±0.040
Cell suspension culture	Leaf	0.390	0.414	0.707	0.524	0.669	0.000	0.134
		±0.012	±0.008	±0.014	±0.007	±0.026	0.000	±0.013
	Stem	0.418	0.141	0.531	0.155	0.758	0.304	0.000
		±0.023	0.017	±0.011	±0.003	±0.056	±0.045	0.000
Callus culture + 50mM NaCl	Leaf	0.287	0.000	0.230	0.171	0.128	0.091	0.143
		±0.006	0.000	±0.012	±0.130	±0.012	±0.005	±0.002
	Stem	0.166	0.000	0.507	0.233	0.153	0.004	0.311
		0.005	0.000	±0.023	±0.024	±0.005	±0.001	±0.031
Callus culture +100	Leaf	0.448	1.390	1.170	0.547	0.825	0.768	0.590
		±0.002	±0.075	±0.003	±0.033	±0.003	±0.002	±0.015
	Stem	0.286	0.000	1.137	0.076	0.109	0.167	0.440
		±0.001	0.000	±0.005	±0.034	±0.006	±0.026	±0.046

Table (6) shows a comparison between tissue culture systems and the intact plant in increased or decreased ratios of the secondary metabolites investigation. Rutin showed an increase in all tissue culture systems except when NaCl was added at 50mM to callus cultures initiated from leaf. This increment was more than doubled (2.578) in cell suspension cultures initiated from stem explants. α-pinene showed an increased ratios in callus cultures initiated from leaf explants (1.030), cell suspension culture initiated from leaf (1.390) and increased to more than four folds (4.670) in callus cultures initiated from leaf and grown on a medium containing 100mM NaCl. Tissue cultures initiated from stem explants mostly showed either reduced levels of α-pinene or not detected. Linalool increased to about 3 folds in callus cultures initiated from leaf explants grown

on a medium containing 100mM NaCl. It is more than doubled in callus and cell suspension cultures initiated from leaf (2.750 and 2.147) respectively. Additionally callus cultures initiated from stem and grown on a medium containing 100mM NaCl showed an increased ratio reaching (1.506). High ratios of geraniol were recorded in callus cultures initiated from leaf explants grown on a medium supplemented with 100mM NaCl, cell suspension cultures initiated from leaf explants, callus cultures initiated from stem explants and grown on a medium supplemented with 50mM NaCl and in cell suspension cultures initiated from stem explants, these ratios were (1.769, 1.697, 1.523 and 1.009) respectively. Apigenin recorded an increase to more than seven folds (7.735) in cell suspension culture initiated from stem explants followed by callus cultures of leaf explants that grown on a medium supplemented with 100mM NaCl that increased to more than 3 folds (3.668), to a lesser extent in cell suspension cultures initiated from leaf (2.973), followed by (1.561) in callus cultures grown on a medium supplemented with 50mM NaCl initiated from stem explants. High ratio of quercetin (2.200) was noticed in cell suspension cultures initiated from stem and callus cultures of leaf explants grown on a medium supplemented with 100mM NaCl (1.829). This followed by (1.208 and 1.184) for the callus cultures initiated from stem explants and grown on a medium supplemented with 100mM NaCl and callus cultures initiated from stem explants respectively. Coumarin in the callus cultures initiated from stem and grown on a medium supplemented with 100mM NaCl showed an increased ratio reached to approximately 1.5 fold (1.477). Similar ratio (1.435) in leaf explants of the same sample was recorded. Callus cultures initiated from stem and callus cultures initiated from stem explants and grown on a medium supplemented with 50mM NaCl showed ratios (1.109 and 1.046) respectively.

The amount of the product may vary depending on the amount of specific supplements such as NaCl in the medium. One benefit of using tissue cultured cells is that they may offer higher metabolites concentrations per cell and synthesis rate than whole plants.

Scott and Dougall (28) reported that the mechanisms by which

these compounds increase may be associated with the amounts of essential enzymes in the biosynthesized pathway or it may be associated with supply of enzyme synthesized precursors.

Dix and Pearce (10), showed the phytochemical effects of nutrient stress in cultured plant cells by the addition of NaCl that caused an increase in secondary metabolites, accumulation of amino acids and other substances in *N. tabacum.* The results disagree with Scott and Dougall (28) who indicated that the plant tissue culture system may produce lower secondary metabolites compared with the intact plant, while Jogdand (15) reported that suspension cultures are ideal for the production of secondary metabolites which are of therapeutic value. His study also confirmed by (27).

Stress affects secondary metabolism of cultured plant cells. One of the features of cultured cells is the activation of genes coding for compounds not usually produced at the whole plant level. This response can occur through the stress mediated induction of specific mRNA. Alternatively, some compounds that are characteristic of the intact plant may not be synthesized (9). Ramawat, (25) reported that the secondary plant products are genetically controlled phenomenon. However, various biotic and abiotic factors influence secondary metabolites production via gene activation or by stimulating the physiological processes leading to enhanced accumulation of such products. Furthermore, the synthesis of most of the secondary metabolites is a several steps reaction involving several enzymes (several genes) which means that the synthesis may be stimulated at any step to enhance their production.

Table 7 represents the total concentration of the studied essential oils (α-pinene, geraniol and linalool) and phenolic compounds (rutin, apigenin, quercetin and coumarin). This table shows that *S. officinalis* essential oils concentration increased in most of tissue cultured systems compared with intact plant. The samples recorded higher concentrations than intact plant since: callus culture initiated from leaves gave (1.323mg/g), cell suspension culture initiated from leaves (1.645mg/g) and callus cultures initiated from leaf and stem explants grown on a medium

supplemented with 100mM NaCl as an elicitor (3.107 and 1.213)mg/g respectively. Phenolic compounds also showed increased concentrations in the *in vitro* systems. They increased in callus cultures initiated from stem explants (0.900mg/g), cell suspension culture initiated from stem explants (1.480mg/g). The sample of callus cultures initiated from both explants and grown on a medium supplemented with 100mM NaCl recorded 2.631mg/g for leaf explants and 1.002mg/g for stem explants.

It was found that in all studied samples, leaf explants showed an increase in essential oils, whereas stem explants showed an increase in phenolic compounds only, except for the sample of callus grown on a medium containing 100mM NaCl which showed increased concentrations of essential oils and phenolic compounds in both stem and leaf explants.

The sample of callus cultures grown on a medium supplemented with 50mM NaCl initiated from both explants showed no increase in essential oils or phenolic compounds. This table indicates that *S. officinalis* contains active compounds in leaf and stem explants but at different concentrations. Nakiboglu (23), Abu-Shanab *et al*.,

(1) and Naghibi *et al*., (22) used leaf explants of *S. officinalis* for extraction of active compounds whereas; Kavvadias *et al*., (17) stated that the medicinal parts of S. *officinalis* are leaves and stems. Various stress factors impact on the qualitative and quantitative accumulation of valuable secondary products in nature. Lila, (18) indicated that elicitors from biotic and abiotic sources can be added to culture media to stimulate secondary metabolites production.

Table (6): Ratios represent the studied secondary metabolites in tissue cultures to the original intac plant.

Source	Explants	Rutin	α-pinene	linalool	Geraniol	Apigenin	Quercetin	Coumarin
Callus culture : Intact plant	Leaf	1.216	1.030	2.750	0.357	0.649	0.640	0.762
	Stem	2.202	0.245	0.000	0.607	0.510	1.184	1.109
Cell suspension culture : Intact plant	Leaf	1.156	1.390	2.147	1.697	2.973	0.000	0.325
	Stem	2.578	0.104	0.703	1.009	7.735	2.200	0.000
Callus culture + 50mM NaCl : Intact plant	Leaf	0.859	0.000	0.698	0.552	0.569	0.217	0.348
	Stem	1.023	0.000	0.671	1.523	1.561	0.029	1.046
Callus culture +100mM NaCl : Intact plant	Leaf	1.326	4.670	3.553	1.769	3.668	1.829	1.435
	Stem	1.763	0.000	1.506	0.496	1.112	1.208	1.477

Table (7): Total concentrations of the studied essential oils and phenolic compounds detected in intact plant and different cultures initiated in vitro.

Source	Essential oil (mg/g)		Phenolic compounds (mg/g)	
	Leaf	Stem	Leaf	Stem
Intact plant	0.936	2.255	1.394	0.696
Callus culture	1.323	0.423	1.138	0.900
Cell suspension culture	1.645	0.827	1.186	1.480
Callus culture + 50mM NaCl	0.401	0.740	0.649	0.634
Callus culture + 100mM NaCl	3.107	1.213	2.631	1.002

CONCLUSION

1. Callus cultures of *S. officinalis* can be induced and maintained on MS medium supplemented with 0.5mg/l kin and 0.05mg/l 2,4-D using stem and leaf explants.
2. Plant tissue culture techniques are potential source for increasing the production of secondary metabolites. Rutin is increased in all tissue culture systems except when NaCl was added at 50mM to callus cultures initiated from leaf.
3. α-pinene production can be increased to more than four folds (4.670) by callus culture initiated from leaf and grown on a medium containing 100mM NaCl, cell suspension culture initiated from leaf (1.930).
4. Linalool production can be increased to about 3 folds in callus cultures initiated from leaf explants grown on a medium containing 100mM NaCl.
5. Geraniol increased to (1.769) times using callus cultures initiated from leaf explants grown on a medium supplemented with 100mM NaCl.
6. Apigenin production can be increased to more than three folds (3.668) using callus cultures of leaf explants grown on a medium supplemented with 100mM NaCl.
7. Quercetin production is increased by (2.2) times in cell suspension cultures initiated from stem and callus cultures of leaf explants grown on a medium supplemented with 100mM NaCl (1.829).
8. Coumarin production can be increased in approximately 1.5 times in callus cultures initiated from stem and grown on a medium supplemented with 100mM NaCl.
9. Essential oils can be produced at higher concentrations using plant

tissue culture systems rather than whole plant.

10. Phenolic compounds can be increased using *in vitro* systems in the callus cultures initiated from leaf and stem explants grown on a medium supplemented with 100mM NaCl.

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Detection of Cholera toxin producing isolate of *Vibrio cholerae* by Radial Immune diffusion method

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ABSTRACT

Cholera toxin was produced and purified from the local clinical isolate of V. cholerae gained from Central Health Laboratories/ Baghdad/Ministry of Health . Few steps was employed for purification of CT including concentration of the protein, back extraction, and gel filtration.

Antiserum prepared from Rabbit immunized with CT conjugated with tween 80-ferrous dextran as a first dose and alum-precipitate as a booster dose and the antibody concentration revealed that CT-Tween 80- ferrous dextran gave high antibody concentration. Radial Immune Diffusion test (RID) showed one precipitate ring between purified CT and antiserum. Also it gave linear relation ship between different CT concentration and antiserum.

INTRODUCTION

Vibrio cholerae is the type species of the genus *Vibrio*, a membere of the family *Vibrionaceae*. This species cause the more mortality and morbidity disease, the cholera.

A major concern early cholera investigator was the differentiated the epidemic associated strains from other atypical vibrios or non cholera vibrios which were regarded primarily as a non pathogenic environmental isolates. Non-O1 strains are part of normal, free living autochthonous bacterial flora in estuarine areas and have not been associated with epidemics but can cause sporadic diarrhea

which had been found to be hypo-toxigenic or non- toxigenic and are ubiquitously distributed in aquatic environment (1,2). Microbiologists have developed isolation techniques, refined the taxonomy and subtyping of vibrios using both the traditional ways based on the phenotypic characters, or the more recent genetics based techniques (3).

Many emerging and reemerging bacterial pathogens synthesize toxins that serve as primary virulence factors and epidemic cholera caused by O1 and O139 which both produce and secrete cholera toxin (CT). Many methods adopted for screening of the presence of CT depending either on the enzymatic activity of CT or its bioactivity saw in animal model, and other methods based on the immunological properties of CT protein. While nucleic acid- based methods appeared to provide more specific, sensitive and speed characterization of CT, so emergence of PCR, multiplex PCR, DNA probes, and DNA sequencing (3).

Despite all the fear in hearing the name cholera toxin, it appeared as a potentially tool for investigating at many researches fields.

Because of the difficulties of CT detection by using animal models and tissue culture. This study came in order to evaluate the immune stimulation of purified CT from local clinical isolate of *V. cholerae* through the preparation of antiserum by new trial and using the antiserum in CT detection and quantification by Radial Immune Diffusion method (RID).

MATERIALS AND METHODS

Bacterial isolates

Clinical isolate of *V. cholerae* O1 belong to ElTor biotype Ogawa was gained from the Central Health Laboratory/ Ministry of Health, identified and characterized again in present study following Elliot (2001) and Otawa (2004).

Production and Extraction of CT

The capability of the isolate of *V. cholerae* O1 to produce Cholera toxin (CT) was measured quantitavely as described by Al-Khafaji (2007). Briefly, overnight

culture of *V. cholerae* grown on 20 ml of production medium (AKI pH 8.5 supplemented with 0.2% of Asparagine and Glucose) was centrifuged at 5000 rpm for 15 minute to prepare free cell extract. Concentration at 80 % salt saturation was done for free cell extract using Ammonium sulfate salt. Finally desalting of concentrated extract was achieved by Sephadex G25.

Measuring of CT

Quantitative measurement of CT was determined by measuring the erythemal activity EA using Guinea pig (8,9).

Toxin Units was calculated (depends on its definition) in that each 5-8 mm of EA is equivalent to 1 Toxin Unit (TU) of enterotoxin.

$TU/ml = (EA\ mm \div 5) \times 10$

Specific toxin activity was calculated as the ratio of TU/ml divided by the protein concentration.

Determination of protein concentration

Protein concentration was measured by dye binding Bradford method using Bovine Serum Albumin for preparing the standard curve (10)Bradford, 1976).

Purification of CT

purification of CT was achieved after extraction, concentration, back extraction between 80% - 20% of salt saturation and gel filtration through Sephadex G100 to obtain purify protein following Stellwagen (1990) and Al-Khafaji (2007).

Cholera toxin antitoxin preparation from Rabbits

Antitoxin against CT was prepared by injecting the Rabbit with purified CT conjugated with ferrous dextran-tween80; booster dose was prepared as aluminium precipitate of CT and given to animals; blood was with drown and serum was obtained and stored at -20°C.

The CT conjugates preparation

CT Ferrous Dextran-Tween mixture was prepared as followed:

purified CT solution of 200 µg/ ml was heated by boiling water bath for 10 minute. Equal volume

of CT solution and Ferrous Dextran – Tween was mixed together and used for rabbit injection.

Booster Dose preparation

To prepare Aluminium precipitate – CT each 2.25 ml of sodium bicarbonate solution was added to each 5 ml of purified CT then 2.25 ml of aluminium potassium sulfate was added slowly with stirring to CT- sodium bicarbonate mixture and left for 15 min with stirring. Finally mixture was spun at 1500 rpm for 15 min, washed for three times with PBS and the formed precipitate was suspended with 5 ml of PBS (12).

Immunization schedule

The immunization time and the location of injection was done following Green and Manson (1998) as presented in table (1)

Table (1) The immunization schedule of Rabbits by purified CT

Dose	Material	Injected vol/ml	Injection location
1 st dose	Ferrous dextran-Tween-CT	1	I.m , thigh muscle
2nd dose	Ferrous dextran-Tween-CT	1	I.m , thigh muscle
3rd dose	Al-CT precipitate	0.25	Marginal ear vein

One week was left between doses for both conjugated method and the first blood collection was done a week after third dose. Another booster doses as Al-CT precipitate (0.25 ml of precipitate suspension) was given via the marginal ear vein and blood was collected 10 day later.

Serum preparation:

Blood was collected by heart puncher, left at room temperature for 1 hour, and at refrigerator for 2-3 hours. Clotted blood was spun at 1500 rpm, serum above precipitate was collected, stored at -20°C and heated at 56°C for 30 min before being used in experiment (12).

Agarose preparation:

Agarose was prepared by weighting and dissolving one gram of agarose on 100 ml of Tris buffer pH 8.8 and sterilized for 5 min (14).

Radial Immune Diffusion agar

Radial immune diffusion was done as shown below:

Ten milliliter of agarose prepared above were mixed with 100, 200, 300 µl of serum prepared above, mixtures were mixed well without bubble formation and poured in petridish.

Dilutions of CT were prepared in PBS to give 240, 210, 180, 120, 90, 60, 30 µg/ ml.

Holes with 3 mm diameter were made in solidified agarose and 50 µl of dilutions were added to holes; plates were incubated at refrigerator for a week and the diameter of precipitate ring was measured. Blot was drown between the precipitated ring diameter and the concentrations (12).

RESULTS AND DISCUSSION

The clinical isolate used in the production of CT was confirmed as *V. cholerae* according to Elliot (2001) and Otawa (2004).

Overnight cultures of the isolate, grown on TCBS medium with yellow color, gave 3-4 mm diameter of circular, smooth, glistening and slightly flattened appearance of colonies. Whereas, it appeared as pale or slightly pink often resembling colonies of late or slow lactose fermenting organisms, with 1-3 mm diameter on MacConkey agar. They appeared as offwhite colour with a dot in their center on the TSA.

The isolate gave positive reaction with oxidase test , positive to string test and cholera red reaction. Fermented glucose not lactose appeared as red surface and yellow bottom of KIA slant with no gas and H_2S formation. The further biochemical tests which must be carried out to confirm the complete characterization as presented in table(2).

Table (2) Biochemical tests for V. cholerae isolate identification

Characters test	The isolate
Growth at pH 5.5	-
Growth at:	
0 NaCl	+
3% NaCl	+
6% NaCl	+
10%NaCl	-
Motility	+
Production of:	
Amylase	+
Gelatinase	+
Hemolycin	+
Lipase	+
Protease	+
MR	+
VP	+
Sensitivity to:	
Polymexin B 50 IU	R
Polymexin B 100 IU	R

+ = Positive results; - -= Negative results; R= Resistant

The recent purification scheme used in CT purification proved that specific activity increased through the purification steps in that salt fractionation by back extraction method led to increasing specific activity from only 62.5 to 117.2 and purification field reached to 1.4. This is due to the exclude of the contaminants from the crude extract. Desalting of the crude concentrated extract with the use of Sephadex G25 led also to increase both specific activity and purification field but CT yield however, highly decreased to reach about only 12% because of the sample dilution that reached to 2.5 fold of dilution factor. The most important advantage was that the specific activity of CT highly increased with gel filtration step on Sephadex G100 to reach 613.4 corresponding approximately 100-

fold the increase in specific activity. The estimation of purification field reached to 9.8 fold (table 3).

Table (3) The purification table of CT

Purification step	Volume (ml)	Protein concentratio (mg/ ml)	Total protein (mg)	EA (mm)	(TU/ml)	Specific toxicity (unit/mg)	Purification field	Yield %
Culture extract	500	0.16	80	5	10	62.5	1	100
20%-80% saturation	20	3.4	68	30*	300	88.2	1.4	30
Desalting	45	0.8	36	12*	120	150	2.4	12
Gel filtration	30	0.326	9.78	20*	200	613.4	9.8	20

*Sample diluted 1:5

The results presented in the purification table (3) showed that CT could be purified with high specific activity by only few steps. Other researchers used gel filtration chromatography as a step in CT purification from *V. cholerae* and its related LT enterotoxin was produced by *E. coli* and enterotoxin from *Clostridium perifringens* (7,15,16,17)

Antiserum preparation against cholera toxin

Polyclonal antibodies are raised by injecting an immunogen into an animal and, after an appropriate time collecting the blood fraction which contain the antibodies of interest.

Several parameters must be considered with respect to the final use to which the antibody will be put including, the specificity of the antibodies in their abilities to distinguish between different antigens, the avidity, and the titer of antibodies which determines the optimal dilution of the antibody in the assay system. Thus we tried in this study the possibility of producing antiserum with high affinity and titer by injecting rabbits with purified whole CT.

The first step in raising the antiserum prepared at the

laboratory is the use of a suitable adjuvant. Dextran-CT-tween tend to serve the adjuvancity of mixtures replacing the complete and incomplete adjuvant usage, because antigens are more immunogenic when presented in an insoluble form or with an adjuvant into which soluble antigen is combined and the mode of action of adjuvant may be attributed to the slow release of the antigen from the mixture (12,14). This treatment employed the use of tween 80 which acts as a lipid droplet because of its chemical nature, it contain hydrophilic and hydrophobic ends. Thus may forming micelles- like structure which encounters the CT as a center and the ferrous dextran serves as hydrophilic polysaccharide conjugate protein (dextran-CT conjugate). This based on the description of the conjugation of CT-B subunit to dextran employed by Bergquist *et al*. (1995) as a model of polysaccharide antigen.

The Radial immune diffusion agar were employed to determine the specificity of antiserum for CT prepared . The results obtained by this study revealed that serum obtained from Dextran- CT- Tween conjugate treatment experiment gave a linearity reaction between the log of antigen (Ag) concentration and the diameter in mm of precipitation ring in single radial immune diffusion reaction (RID).

Figure (1) Precipitating ring between purified CT and antiserum in Radial Immune Diffusion test

Each concentration showed only one precipitation ring around well which determine the identity of the preparation of Ag solution as presented in figure (1). Our results in accordance with Finklestein and Lopsolloto (1970) how, used RID in measuring the CT quantity through the purification steps of CT.

The emergence of using a purified CT in preparing antiserum from different animals to overcome the unspecific is related to different antigenic determinant which are possibly there with the antigen of interest. On the other hand, the whole CT as well as its B- subunit and their related LT produced by *E. coli* and other bacteria were very immunogenic substances. However, the differences between the immunogenicity of the A and B-subunits, in that the immunization with intact toxin which gives rise to antisera, always react strongly with B-subunit but only irregularly and at lower titer with A-subunit. Also B-subunit is much more immunogenic than A- subunit, although it is less potent than the intact toxin molecule (19,20,21,22). Moreover, the Many methods were adopted to make standardization of antitoxin preparation as titration of antitoxin neutralization of skin permeability factor, loop test , Chinese hamster ovary cell assay (CHO) cell assay and the estimation of vibrocidal titer in which all need either laboratory animals or sophisticated facilities and equipment (19). Thus the precipitation in gel by RID is an easy technique to examine the specificity of the test sera against CT

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Antioxidant and Lipid Peroxidation Level in Patients with Breast Cancer

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ABSTRACT

The extent of lipid peroxidation as evidenced by the formation of thiobarbituric acid react ive substances (TBARS) and the antioxidants superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx) and glutathione Stransferase (GST) in serum samples of 53 breast cancer patients in and around Santa Maria Rs, Brasil , were studied. Controls consisted of members of the public with no previous history of breast cancer or other cancer-related diseases. The plasma samples of the breast cancer patients showed enhanced level of lipid peroxidation when compared to the corresponding controls. This was accompanied by a significant elevation in both enzymic and non-enzymic antioxidants. These findings indicate the significant increase in lipid peroxidation as evidenced by the level of TBARS and antioxidant status such as elevated SOD, CAT, GPx, GSH and GST in samples from breast cancer patients compared to controls.

Keywords : *antioxidants , breast cancer, glutathione peroxidase, lipid peroxidation*

INTRODUCTION

Breast carcinoma is one of the most common neoplasms in women and is a leading cause of cancer-related deaths world wide. The aetiology of breast cancer is multifactorial. Significant breast cancer risk factors include age, early age at menarche, late age of menopause, late age at first pregnancy, obesity, oral contraception, hormone replacement therapy, diet, family history, lactation and prior history of benign breast disease.(1) In the United States, breast cancer is one of the most common malignant tumours in women. The American Cancer Society estimated that approximately 210,000 new patients would be diagnosed with breast cancer and 40,000 women would die from this disease in 2004.(2). During five-year period (2000-2004) 63923 Iraqi patients, with various types of newly diagnosed cancer were registered by the Iraqi Ministry of Health from all Iraqi provinces with exception of 3 Northern provinces (Sulaimanyia, Erbil, and Dohouk). 32281 patients were males (50.5%) and 31652 patients were females (49.5%).Breast was by far the most common site of cancer accounted for 16% of all Iraqi patients(3) The common risk factor in the development of breast cancer is the increased lifetime exposure to endogenous or exogenous estrogens. A number of genes, including BRCA1 and BRCA2, HER-2/neu and p53, have been linked to breast cancer susceptibility and development.(4) Oxygen-free radicals (OFR) generated by a number of processes *in vivo* are highly reactive and toxic.(4) However, biological systems have evolved an array of enzymic and non-enzymic antioxidant defense mechanisms to combat the deleterious effects of OFR. Superoxide dismutase (SOD) and catalase (CAT) play a key role in the detoxification of superoxide anion and hydrogen peroxide (H2O2), respectively, thereby protecting against OFR-induced damage. Reduced glutathione (GSH) in conjunction with glutathione peroxidase (GPx) and glutathione S-transferase (GST)

plays a central role in the defense against free radicals, peroxides and a wide range of xenobiotics and carcinogens.(5,6) Oxidative stress arises when there is an imbalance between OFR formation and scavenging by antioxidants. Excess generation of OFR can cause oxidative damage to biomolecules resulting in lipid peroxidation, mutagenesis and carcinogenesis. OFR-induced lipid peroxidation has been implicated in neoplastic transformation.(7) Although a number of studies have un reveled the role of estrogens as well as the imbalance in oncogenic and tumor suppressor genes in breast cancer, the involvement of oxidative stress in breast carcinogenesis has not been extensively documented. We therefore examined the extent of lipid peroxidation, as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) and the status of the antioxidants SOD, CAT, GSH, GPx and GST in the plasma of patients with carcinoma of the breast.

METHODS

53 newly-diagnosed breast cancer patients from Santa Maria , Brazil, with a mean age of 46.71 ± 3.85 years, and who had not undergone any previous treatment for their tumors, were chosen for the study. The patients were clinically categorized as stage II (25 patients) and stage III (28 patients) infiltrative ductal carcinoma of the breast. The patients were not using hormones, oral contraceptives and were nonsmokers. None of them had concomitant diseases such as diabetes mellitus, liver disease and rheumatoid arthritis (Table I). Informed consent was obtained from

all the participants. The Human Ethics Committee, Brazil approved the study. Controls consisted of members of the public with no previous history of breast cancer and other cancer-related diseases.

Blood was collected by venous arm puncture in patients and controls. The collected blood was injected into EDTA vaccutainets and the plasma was separated by centrifuging at 1,000 g for15 minutes. All the chemicals

and reagents used in the study were of analytical grade and purchased in Hi-Media Laboratories (Brazil) and Sigma (St Louis, MO, USA). Lipid hydroperoxides were estimated by the method of Jiang et al.(8) The reaction mixture in a total volume of 2.0 ml containing 0.2 ml of plasma and 1.8 ml of Fox reagent, was incubated for 30 minutes. Hydroperoxides are detected by their ability to oxidize ferrous iron, leading to the formation of a chromophore with an absorbance maximum at 560 nm. Lipid peroxidation was estimated by measurement of thiobarbituric acid reactive substances (TBARS) in plasma by the method of Yagi.(9) The pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde, a secondary product of lipid peroxidation was estimated at 532 nm. GSH was determined by the method of Ellman.(11) GSH estimation was based on the development of a yellow color when 5,5-dithio (2-nitrobenzoic acid) was added to compounds containing sulfhydryl groups. GPx activity was assayed by the method of Rotruck et al(12) with modification. A known amount of enzyme preparation was incubated with H2O2 in the presence of GSH for a specified time period. The amount of H2O2 utilized was determined by the method of Ellman.(11) The activity of GST was estimated by the method of Habig et al,(13) by following the increase in absorbance at 340 nm using 1-chloro-2,4-dinitrobenze as substrate. SOD was assayed by the method of Kakkar et al(14)

Table I. General characteristics of breast cancer patients.

General characteristics	Breast cancer patients
Total number of subjects	**53**
Age range (years)	20–70
Menopausal status	
Premenopausal	28
Postmenopausal	25
Cancer site	

Left breast	20
Right breast	33
Clinical status	
Infiltrative ductal carcinoma	53
Clinical stage	
Stage II T2N1M0	28
Stage III T3N1M0	25

T: tumor size; T1: < 2 cm, T2: 2–4 cm, T3: > 4 cm.
N: nodal metastasis, N0: no regional lymph node metastasis,
N1: metastasis in a single ipsilateral node of < 3 cm diameter,
M: distant metastasis, M0: no distant metastasis.

Based on the 50% inhibition of the formation of nicotinamide, adenine dinucleotide (NADH)-phenazine methosulfate-nitroblue tetrazolium formazan at 520 nm, Hemoglobin in the haemolysate was measured according to the method of Drabkin and Austin.(15). Blood was diluted in an alkaline medium containing potassium cyanide and potassium ferricyanide. Haemoglobin oxidized to methaemoglobin combines with cyanide to form cyanmethaemoglobin which was measured at 540 nm. The data for biochemical analyses are expressed as mean and standard deviation (SD). Statistical comparisons were performed by Student's *t*-test using the Statistical Package for Social Sciences version 10.0 (SPSS Inc, Chicago, IL, USA).

RESULTS

The extent of lipid peroxidation in the breast cancer samples as evidenced by the formation of TBARS, and lipid hydro peroxides, is represented in Table II.
The concentration of TBARS in the cancer samples was significantly higher ($p < 0.05$) when compared to the corresponding control samples. Values and lipid hydroperoxides showed a similar but significantly greater response

($p < 0.05$) in cancer samples compared to the corresponding control samples.

Table II. Lipid peroxidation in breast cancer patients (mean ± SD, n = 53).

Parameters	Controls	Breast cancer patients
TBARS (nmol/100 mg protein)	118.58 ± 10.42	142.26 ± 11.44*
LOOH (nmol/100 mg protein)	0.35 ± 0.06	0.63 ± 0.04*

*As compared with breast cancer controls, $p < 0.05$.

Table III. Antioxidant status in breast cancer patients (mean ± SD, n = 53).

Parameters	Controls	Breast cancer patients
SODa	18.13 ± 1.40	31.14 ± 1.35*
CATb	7.51 ± 0.65	12.35 ± 0.75*
GSHc	8.95 ± 0.39	19.35 ± 0.16*
GPxd	14.71 ± 0.91	28.20 ± 1.31*

a. Amount of enzymes required to give 50% inhibition of NBT reduction/ mg protein.

b. µmol H2O2 utilised/s/ mg protein.

c mg/di plasma

d µmol GSH utilised/min/mg protein.

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*As compared with breast cancer control. p < 0 .05.

Table III indicates the antioxidant profile in the cancer plasma samples. The concentration of GSH and the activities of SOD,

CAT, GPx and GST in the cancer samples showed a marked elevation compared to the controls. The respective concentrations of SOD and CAT were significantly increased compared to the controls ($p < 0.05$). The activity of GPx was 1.50 - fold higher in breast tumours compared to the controls. GSH and GST exhibited nearly a 2–3-fold increase ($p < 0.05$) in the patient plasma samples compared to the corresponding controls.

DISCUSSION

Damage to the breast epithelium by OFR can lead to fibroblast proliferation, epithelial hyperplasia, cellular atypia and breast cancer. Studies have shown increased lipid peroxidation in solid tumors.(16,17) Tamoxifen therapy in post menopausal women with breast cancer reduced the increase in lipid peroxidation. Damage to the mammary epithelium by reactive oxygen species can lead to fibroblast proliferation epithelial hyperplasia, cellular atypia and breast cancer.(18,19) The increase in plasma lipid peroxidation in breast cancer seen in the present study was associated with enhanced antioxidant capacities. Increased generation of OFR, such as O2 and H2O2, can induce SOD and CAT. An increase in total and mitochondrial SOD activities due to over expression has been reported.(20) Increased SOD mRNA expression was observed in cancer samples from patients with carcinoma of the breast.(21) Higher activity of CAT has been documented in tumor cell lines compared to controls.(22) Our results lend credence to these reports. Glutathione, an important substrate for GPx and GST, has been documented to have regulatory effects on cell proliferation.(23) Over expression of GSH has been reported in both animal and human tumors by us as well as by other workers.(17,24,25). A significant increase in the activity of GPx, the first step of enzyme defense against H2O2 and other hydro peroxides, has been reported in tumors.(26,27) The higher activity of GPx in breast cancer cell lines was suggested to result from an

increased expression of genomic DNA.(28) GST, which is involved in the detoxification of electrophilic toxins and carcinogens, is increased in most of the human tumors studied. High concentrations of GST may rapidly detoxify anticancer agents, thereby preventing their cytotoxic action. Enhanced GST activity in breast cancer samples in our study supports ubiquitously-reported induction of GST, especially the iso-enzyme GST-P in various cancer tissues and cell lines.(29,30) Overproduction of OFR coupled with antioxidant depletion is recognized to result in oxidative stress.(5,6) The increase in lipid peroxidation in breast cancer patients in the present study was counter balanced by enhanced host antioxidant defense systems protecting against oxidative stress. Recent reports suggest that oxidative stress can cause up regulation of antioxidant enzymes that render cells more resistant to subsequent oxidative insult.(31) Prolonged exercise generates oxidative stress, which results in increased endogenous antioxidants. Exercise-trained mice showed increased levels of hepatic SOD and CAT.(32,33) Several researchers reported decreases in the antioxidant level and increases in the lipid peroxidation level.(1,2) Over expression of antioxidants has been documented in a wide variety of malignant tumors, including breast cancer.(17,27,29) Cancer cells with increased activities of antioxidant enzymes are presumed to escape recognition by cytotoxic lymphocytes.(34) From the results of the present study, we suggest that increased lipid peroxidation and host antioxidant defenses associated with the development of breast cancer may offer a selective growth advantage to tumor cells over their surrounding normal counter parts.

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Detection of Hepatitis B Virus in a Group of Iraqi Pregnant Women

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ABSTRACT

This is cross-section study conducted in Iraq for pregnant women during the period of September-November 2007. The main objective of the present study is to estimate the prevalence and identify the risk factors of the hepatitis B infection among pregnant women.
The study population includes a hundred pregnant women aged from 15-41 years. They came from different parts of rural and urban areas. An enzyme-linked immunosorbent assay (ELISA) was used for estimation of Hepatitis B surface antigen (HBsAg), while MiniVidas was used for estimation of Hepatitis B core Antibody (IgG & IgM).

Our findings included the prevalence of chronic HBV infection among pregnant women is 3(3%) and previous HBV infection was 2 (2%) giving an overall prevalence of HBV infection of 5(5%). Hepatitis B markers showed HBsAg was positive in 3 (3%), HBcAb (IgG) was positive in 5 (5%) while HBcAb (IgM) was absent in the population of the study.

Those with HBV infection tended to be older age range between 30-39 years. Also we found that prevalence of HBV infection is 2(4.4%) for HBsAg, 4(8.9%) for each of HBc IgG.

In present study, the most important risk factors for pregnant women are blood transfusion, intravenous drug injecting, and husband hepatitis.

Also we detected the most occurrence of HBV infection was in

unvaccinated pregnant women with HBV vaccine.

INTRODUCTION

Hepatitis is an ancient disease descried as a disorder in which viruses or other mechanisms produce inflammation in the liver cells, resulting in their injury or destruction. In most cases, this inflammatory process is triggered when the immune system fights off infection caused by viruses. It can also be caused, however, by an overactive immune system that attacks its own liver cells. Inflammation of the liver can also occur from medical problems, drugs, alcohol, chemicals, and environmental toxins. Hepatitis varies in severity ranged from a self-limited condition with total recovery to a life threatening or life-long disease [(1)]. Hepatitis is one of two ways:

- Short-term (acute hepatic):

Acute hepatic can begin suddenly or gradually, but it has a limited course and lasts beyond one or two months. Usually, there is only spotty liver cell damage and evidence of immune system activity, but on rare occasions, acute hepatitis can cause severe, even life-threatening, liver damage [(2)].

- Prolonged (chronic hepatitis):

The chronic forms of hepatitis persist for a prolonged period. Experts usually categorize chronic hepatitis as either (1) chronic persistent or (2) chronic active hepatitis, which are indications of severity. Chronic persistent hepatitis is usually mild and non- progressive or slowly progressive, causing limited damage to the liver. If damage to the liver is extensive and cell injury occurs beyond the portal tract, chronic active hepatitis can develop [(2)].

The term viral hepatitis is often thought to be synonymous with disease caused by the known hepatotropic viruses, including hepatitis.
Viruses A, B, C, D, E, and G .However, the term hepatotropic are itself a misnomer. Infections with hepatitis viruses, especially hepatitis viruses B and C, have

been associated with a wide variety of extra hepatic manifestation. Infrequent causes of viral hepatic include adenovirus, cytomegalovirus, Epstein-Barr virus, and rarely, herpes simplex virus infection [3, 4].

Viral hepatitis represents an important health hazard, since the earlier age at which the infection is acquired, the greater risk to develop a serious consequence [5, 6] .Viral hepatic is common on worldwide [7] . It causes millions of death among the population of the world yearly [8] .It is estimated that more than 1/3 of the total population of the world has been exposed to hepatitis viruses [9,10] . Worldwide about 350 million persons are suffering from chronic hepatitis B infection [11] .The risk for them to develop hepatocellular carcinoma is about 100-200 times higher than that for non-chronic carriers .Chronicity occurs in 90% of the patients with parental transmission and 5-10% when HBV acquired during adulthood[12]. While for HCV the number of infected persons , who are considered as chronic carriers is about 2.7-3.5 million cases world widely [5, 13, 14, 15], chronicity occurs in 80-85% of the infected patients[5, 16, 17]. HBV kills about 1 million persons each year [18], and 15-25% die prematurely [13, 17], also it was estimated that hepatitis viruses' infections have high prevalence in East Asia [19].

In the United States, viral hepatic, in general, ranks third among reportable communicable diseases. Every year more than 600 000 Americans become newly infected with some form of viral hepatitis, yet only 10% of these cases are reported to the health authorities. [20,21].

Viral hepatitis and pregnancy

Viral hepatitis is the most common cause of jaundice in pregnancy [22, 23]. The course of most viral hepatitis infections is unaltered by pregnancy [23, 24]. However, a severe course of viral hepatitis in pregnancy has been noted in patients with hepatitis E and disseminated herpes simplex virus (HSV) infections [23, 24] .

In the United States, 15 000 pregnant women who are hepatitis B surface antigen (HBsAg) – positively deliver annually [24]. Universal screening of pregnant women for HBsAg is now performed to reduce parental transmission of hepatitis B virus [25].The risk of hepatitis B virus vertical transmission is 10% in mother with negative HBeAg and positive HBeAb and 90 % in those with positive HBeAg [24, 25, 26]. The risk of chronic hepatitis virus's infection in neonate who does not receive immunoprophylaxis and vaccination for hepatitis B virus is 40 % [25].

Infants of HBsAg positive mother should receive hepatitis B immune globulin immunoprophylaxis at birth and hepatitis B vaccine at one week, one month and six months after birth [24, 25]. This regimen reduces the incidence of hepatitis B virus vertical transmission to 0-3% [24].

In case of acute hepatitis B virus infection complicating pregnancy, the prevalence of neonatal infection depends on the time during gestation that maternal infection occurs [27]. Neonatal hepatitis B virus infection is rare if maternal infection takes place in the first trimester. The infection occurs in 6% of neonates of women infected in the second trimester, in 67% of those infected in the third trimester and in virtually all of those infected in the immediate postpartum period [25].

MATERIALS AND METHODS

The study population includes 100 pregnant women aged from 15-41 years. These women belong to rural and urban areas.

After completing the interview, a blood sample was drawn from each subject included in the study. About 5 ml of blood was obtained by vein puncture under septic conditions. The blood was collected in plain tubes without anticoagulant. Each tube was labeled by a code number. Sera were separated and stored at -20 C^o until tested for HBV.

Three serological tests were done for each blood sample as follows:

1. Qualitative determination of Hepatitis B surface Antigen (HBs Ag) by ELISA, using commercial kits obtained from Hepanostica HBsAg ultra (bioMerieux. Netherlands). 192-test kit.
2. MiniVidas for detection of HBc IgM (VIDAS HBc IgM 11). BioMerieux-France.
3. MiniVidas for detection of HBc IgG (VIDAS HBc IgG 11), bioMerieux-France.

Instruments

Instruments used during the study are listed in bellow:

1- Centrifuge, K24, 12x10 ml (Janetzki , Germany) .

2- Micro well system reader 2305 (Organon Teknika, Austria) .

3- MiniVidas system (BioMerieux S A, France) .

For HBsAg :

1. A 25 µl specimen diluents was Pipette into the assigned well.
2. A 100 µl (undiluted) sample or control was Pipette into assigned wells which include three negative controls and one positive control in each micro plate (pipette the controls after the samples).
3. Plates were covered with an adhesive seal & incubated for 60 minutes, (+ or -) 5 minutes.
4. The content of the wells was aspirated and filled completely (approximately 100 µl) with the diluted washing solution (phosphate buffer).

   Note: the phosphate buffer concentrate was diluted 25 times with distilled water, and the contents of the buffer concentrate (100 ml) were poured in a flask and filled up to 2500 ml with D.W. It was mixed well before use. Phosphate buffer is stable for two weeks at 2-8 C^{o}.
5. The aspiration-washing procedure was repeated three more times, after the last wash was plated, the micro well plate was blotted on absorbent tissue to remove any excess liquid from the wells.
6. A 50 µl conjugate solution was pipette into each well except those used for blank.

7. The plate was covered with an adhesive seal and incubated at 37 C^{o} for 60 minutes (+ or -) 5 minutes.
8. During the last 5-10 minutes of second incubation period, Tetramethyl benzidine (TMB) substrate prepared by combined the required amount of TMB solution with an equal volume of urea peroxide solution in a new disposable vial depending on the number of wells being run. Mixed well. TMB substrate must be almost colorless when used.
9. The adhesive plate cover was removed and discarded. The micro well plate was washed with phosphate buffer as in step 4.
10. A 100 µl of TMB substrate was Pipette into each well. Prevented mix or shake. Any TMB substrate that has been stored beyond the indicated period of use was discarded.

The micro well plates were incubated at 15-30°C for 30 minutes in the dark.

The reaction was stopped by adding 100 µl of (stopping solution) 1N sulphuric acid .

11. The reader was blanked (the spectrophotometer) at 450 nm with the blank well & the absorbance was read for each well within 15 minutes.

For HBcAb (IgM & IgG) :

1- Before each new lot of reagents is used, factory master calibration curve date was entered into the MiniVidas using the master lot entry Master lot entry (MLE) card included in each kit.

2- For IgM, we used one Hepatitis B core IgM (HBcM) strip and one HBcM Solid Phase Receptacle (SPR) for each sample, control or calibrator to be tested. While for IgG, we used one Hepatitis B core IgG (HBcG) strip and one HBcG SPR.

3- From menu of instrument (MiniVidas), we selected the HBcM or HBcG to enter the test code and indicate the number of determinations to be performed. The calibrator

must be identified by Standard 1(S1) , Standard 2 (S2), positive control by C1, and negative control by C2.

4- The calibrator, controls and samples were mixed by using a vortex type mixer.

5- Pipette 100 µl of calibrator, control, and sample into the sample wells.

6- In the next step, we inserted the Solid phase Receptacle (SPRs) and strips into the instrument.

7- Initiated the assay as directed in the operator's manual.

All the assays steps were performed automatically by the instrument. The assay was completed within approximately 55 minutes.

Determination of HBsAg by ELISA

The presence or absence of HBsAg in the samples analyzed was determined by relating the absorbance value of each sample to the cut-off value of the mean absorbance value of the negative control (NCx) plus 0.040 .

Cut-off = NCx + 0.040

DISCUSSION

* A negative result indicates that the sample tested does not contain HBsAg.
* A positive result indicates that the sample tested contain HBsAg.
* Specimens that initially show a positive result should be retested in duplicate. If the specimen is positive in one or both retests, additional testing including confirmatory testing, should be performed before specimen is considered positive for HBsAg .

<u>The characteristics of the study population</u>

The whole number of the population study is 100 pregnant women aged from 15-41 years (mean =26.59, std. deviation =5.343). The majority 59(59%) of pregnant women are between 20 -29 years of age, 30(30%) were between the age of 30-39 years,8(8%) were less than 20 years old, while only 3(3%) were more than 39 years old . More than half, 61% are living in urban

areas while 39(39%) were living in rural areas. Table (1).

Table 1: Characteristics of population study (pregnant women) Including Age, Place of residence, and Educational		
Variable	No.	%
Age		
< 20	8	8
20-29	59	59
30-39	30	30
> 39	3	3
Total	100	100
Place of residence		
Rural	39	39
Urban	61	61
Total	100	100

The prevalence of hepatitis B infection among pregnant women

The overall serological profile of hepatitis B infection among pregnant women is presented in Table (2). Hepatitis B markers :HBsAg was positive in 3 (3%) , HBcAb (IgG) was positive in 5(5%) while HBcAb (IgM) was absent in population study .

Table 2: The results of the prevalence of hepatitis B markers among
Pregnant women

Markers	Result	Pregnant women(n=100)	
		Number	percent
HBsAg	Positive	3	3
	Negative	97	97
HBcAb(IgM)	Positive	0	0
	Negative	100	100
HBcAb(IgG)	Positive	5	5
	Negative	95	95

The prevalence of HBV infection among pregnant women according to age.

As shown in Table (3), the prevalence of HBsAg infection was higher among those between 20-29 and 30- 39 years of age .About 59 (59%) of the total number of pregnant women had age of 20-29 years old, 2 (3.4%)of them had positive serology for HBsAg while about 30 (30%) of total number of population study had age 30-39 years old, 1(3.3%)of them had positive serology for HBsAg. While no prevalence HBsAg infection among those less than 20 years and more than 39 years of age.

Table 3: The prevalence of HBsAg among pregnant women according to age

Age (Years)	HBsAg					
	Positive serology		Negative serology		Total	
	Number	Percent	Number	Percent	Number	Percent
<20	0	0	8	100	8	100
20-29	2	3.4	57	96.6	59	100
30-39	1	3.3	29	96.7	30	100
>39	0	0	3	100	3	100
Total	3	3	97	97	100	100

Table (4), shows the prevalence of HBc IgG infection was higher among those between 20-29 and 30- 39 years of age .About 59 (59%) of the total number of pregnant women had age of 20-29 years old,3 (5.1%) of them had positive serology for HBc IgG while about 30 (30%) of total number of population study had age 30-39 years old, 2(6.7%)of them had positive serology for HBc IgG. While no prevalence HBc IgG infection among those less than 20 years and more than 39 years of age.

Table 4: The prevalence of HBc IgG among pregnant women according to age						
Age (Years)	HBc IgG					
	Positive serology		Negative serology		Total	
	Number	Percent	Number	Percent	Number	Percent
<20	0	0	8	100	8	100
20-29	3	5.1	56	94.9	59	100
30-39	2	6.7	28	93.3	30	100
>39	0	0	3	100	3	100
Total	5	5	95	95	100	100

There were a significant differences in the prevalence of HBV and HCV infection among pregnant women of different age groups ($p < 0.05$) .

The prevalence of HBV infection by place of residence:

Tables (5, 6) demonstrate that 1(2.6%) of the pregnant women who were living in rural areas had positive serology for HBsAg, and 3(7.7%) of the pregnant women were showed positive serology for each of HBc IgG . While 2(3.3%) of those living in urban areas had positive serology for each of HBsAg, HBcAb (IgG). The difference, however, did not reach the statistical level of significance (p value > 0.05).

Table 5: The prevalence of HBsAg among pregnant women according place of residence						
Place of residence	HBsAg					
	Positive serology		Negative serology		Total	
	Number	Percent	Number	Percent	Number	Percent
Rural	1	2.6	38	97.4	39	100
Urban	2	3.3	59	96.7	61	100
Total	3	3	97	97	97	100

Table 6: The prevalence of HBc IgG among pregnant women according place of residence						
Place of residence	HBc IgG					
	Positive serology		Negative serology		Total	
	Number	Percent	Number	Percent	Number	Percent
Rural	3	7.7	36	92.3	39	100
Urban	2	3.3	59	96.7	61	100
Total	5	5	95	95	100	100

The prevalence of HBV infection in relation to selected risk factors:

1- The history of previous blood transfusion

A total of 10 (10%) of pregnant women had a history of blood transfusion, 2 (20%) of them were found to be positive for HBcAb (IgG). While only 3 (3.3 %) of those without a history of blood transfusion had a positive serology for each of HBsAg & HBc IgG .Table (7). There is a significant statistical association between previous blood transfusion and HBc IgG ($p<0.05$), but this association was not detected between such a history & each of HBsAg ($p > 0.05$).

Table 7: The Prevalence of HBV infection among pregnant women in relation to previous blood transfusion							
Serological test	Result	Blood transfusion					p.value
		Yes		No		Total	
		No.	%	No.	%		
HBsAg[1]	Positive	0	0	3	3.3	3	> 0.05
	Negative	10	100	87	96.7	97	
	Total	10	100	90	100	100	
HBc IgG[2]	Positive	2	20	3	3.3	5	< 0.05
	Negative	8	80	87	96.7	95	
	Total	10	100	90	100	100	

(1) $x^2 = 0.344$, df = 1 , p value = 0.727 (2) $x^2 = 5.263$, df = 1 , p value = 0.049

2-The history of intravenous drug injecting

A total of 53 (53%) of the total population study has a history of previous intravenous drug injecting , 3 (5.7%) of them had positive serology for HBsAg , 5 (9.4 %) for HBc IgG . While positive serology was absent among those with no such a history. The HBc IgG Ab positive serology showed a significant association with the previous intravenous drug injection ($p < 0.05$) Table (8).

Table 8: The Prevalence of HBV infection among pregnant women in relation to intravenous drug injecting							
Serological test	result	intravenous drug injecting					p.value
		Yes		No		Total	
		No.	%	No.	%		
HBsAg[1]	positive	3	5.7	0	0	3	> 0.05
	negative	50	94.3	47	100	97	
	Total	53	100	47	100	100	
	positive	5	9.4	0	0	5	

HBc IgG[2]	negative	48	90.6	47	100	95	< 0.05
	Total	53	100	47	100	100	

(1) x^2 = 2.743 , df = 1 , p value = 0.145 (2) x^2 = 4.667 , df = 1 , p value =0.038

3- The history of husband hepatitis

A total of 4 (4%) of pregnant women have a history of husband hepatitis, 2 (50%) of them show a positive serology for HBsAg, 2(50%) were positive for HBc IgG. On the other sites, 1(1%) of pregnant women has no previous history of husband hepatitis showed positive HBsAg, 3(3.1%) for HBc IgG. There is a significant statistical association between such history and each of HBsAg & HBcAb IgG ($p < 0.05$) Table (9).

Table 9: The Prevalence of HBV infection among pregnant women in relation with husband hepatitis

Serological test	Result	Husband hepatitis					p.value
		Yes		No		Total	
		No.	%	No.	%		
HBsAg[1]	Positive	2	50	1	1	3	< 0.05
	Negative	2	50	95	99	97	
	Total	4	100	96	100	100	
HBc IgG[2]	positive	2	50	3	3.1	5	< 0.05
	negative	2	50	93	96.9	95	
	Total	4	100	96	100	100	

(1) x^2 = 31.629 , df = 1 , p value = 0.004 (2) x^2 = 17.763 , df = 1 , p value = .011

The prevalence of hepatitis B infection in relation to history of vaccination:

The prevalence of hepatitis B infection is higher among pregnant women with no history of HBV vaccination. Table (10) showed that 0(0%) of vaccinated pregnant women were positive for HBsAg and HBc IgG, while 3(5.4%) & 5(8.9%) of pregnant women with no

history of vaccination showed positive serology for each of HBsAg and HBc IgG respectively. There is significant statistical association between HBV infection and non vaccinated pregnant women ($p < 0.05$).

Table 10: The Prevalence of HBV infection among pregnant in relation with history of vaccination							
Serological test	result	history of vaccination					p.value
		Yes		No		Total	
		No.	%	No.	%		
HBsAg[1]	positive	0	0	3	5.4	3	< 0.05
	negative	44	100	53	94.6	97	
	Total	44	100	56	100	100	
HBc IgG[2]	positive	0	0	5	8.9	5	< 0.05
	negative	44	100	51	91.1	95	
	Total	44	100	56	100	100	

(1) $x^2 = 3.552$, df = 1 , p value = 0.049 (2) $x^2 = 4.135$, df = 1 , p value = 0.046

HBcAb (IgM)

Because there is no case of HBcAb (IgM) among population study, it is difficult to find any association with independent variables.

CONCLUSION

Viral hepatitis is one of the important infectious diseases , which represent an important health hazard worldwide . HBV is among the world's most wide-spread infectious agent causing million of hepatitis cases and deaths each year [8]. Large percents of the infected individuals develop chronic sequels [28, 29, 30]. Comprehensive understanding of the epidemiology & ecology of viral hepatitis is important for devising successful control measures of the disease [28, 29].

Thus, this cross sectional study was carried out in order to determine the prevalence of hepatitis B among pregnant women.

Limitation of the screening methods

This study was carried out over a period of three months in which, 100 subjects underwent four investigations namely HBsAg, HBcAb (IgM), HBcAb (IgG), . However other hepatitis B markers which include: Anti hepatitis B surface antibody, HBe antigen and antibody were not included because the kits for these markers were not available in Iraq at the time of the study.

ELISA was adopted for the detection of hepatitis B surface antigen. ELISA is the most useful and sensitive method for the detection of HBsAg [31] while MiniVidas was used for the detection of HBcAb (IgM & IgG).

The appearance of HBsAg is the first evidence of HBV infection, appearing before biochemical evidence of liver disease and it persists throughout the clinical illness. Persistence of HBsAg after the acute illness may be associated with clinical and laboratory evidence of chronic hepatitis for variable period of time, its detection establish the infection with HBV & implies infectivity, i.e. detection of HBsAg indicates both a recent (acute) or persistence (chronic) infection. It is positive in the following situations : Acute hepatitis B , Chronic hepatitis B with viral replication

and chronic hepatitis B with low viral replication[31].

The prevalence of hepatitis B infection

In this study, the prevalence of sero-positive for HBsAg is (3%), HBcAb (IgM) is (0 %), and HBcAb (IgG) is (5%). Total seropositivity {i.e. HBsAg + HBcAb (IgM & IgG) }rate for HBV infection obtained from this study are (5%), indicating that up to (3%) of the population has chronic infection and (2%) of the pregnant women under investigation are found to be previously infected with HBV.

In other studies, for prevalence of HBV infection among pregnant women was reported as (3.8%) for HBsAg in Romania by Geza,et al.2004 [32], (9.3%) for HBsAg in Nigeria by Christy, et al. 2004 [33] ,(3.8%) for HBsAg & (13%) for HBcAb in Venezuela by Pujol, et al. 1994 [34], (2 %) for HBsAg & (4 %) for HCV in Pakistan by Chotani, et al. 2004 [35] , (1%) for HBsAg & (1.9%) in Italy by Vencenzo, et al. 2000 [36],(2%) for HBsAg in North Jordan by Kkoloud.1995[37], (2.8%) for HBsAg in Greece by Papaevangelon, et al.2006[38], (1.7%) for HBsAg & (4.2%)for HBcAb in Brazil by Bertolini, et al.2006[39] , (0.60 %) for HBsAg in USA by Gary, et al. 2003 [40] , (2.44 %) for HBsAg in Saudi pregnant women by Khalil, et al.2005[41] & AL-Mazrou, et al. 2004[42], (9.3 %)for HBV in Zambia by Gilbert S.2006[43] ,(13%) for HBsAg in Taiwan by Ho-Hsiung, et al. 2003[44].

In our study, we found that the highly prevalence of hepatitis B infection may be partly due to:

1- Previous economic blockade.
2- Poor supplement of the health equipment.
3- Unavailability of the vaccine.
5- Poor environmental condition of people and infections preventive measures.

The prevalence of HBV infection among pregnant women in association with age.

The prevalence of HBV infection was slightly higher among pregnant women between 20-29 year and 30-39 years of age because this period of age is the reproductive

age for married women. Most results were obtained in Nigeria by Christy,et al.2004(33), in Brazil by Bertolini,et al. 2006(39), in Saudi Arabia by Khalil, et al.2005(41) and in Taiwan by Ho-Hsiung, et al. 2003(44) , they found the prevalence of HBV infection in pregnant women with age group 20-29 year and 30-39 years .Our findings are similar to other studies done by Stevens, et al.1990 in USA (45) , Deseda, et al.1990 in Puerto Rico (46), Paba, et al.1999 in Nigeria (112)47 , Ashok, et al, 2007 in India (48) and Prasad, et al. 2007 in Switzerland (49).

The prevalence of HBV infection among pregnant women in association with place of residence

In the present study, there is no significant statistically association between place of residence and the prevalence of hepatitis B infection ($p>0.05$). This finding is in agreement with those recorded in Pakistan by Chotani, et al.2004 (35), in Italy by Vencenzo, et al.2000 (36,) in North Jordan by Kkoloud.1995(99)37,in Greece by Papaevangelon, et al. 2006(38) in Brazil by Bertolini, et al. 2006 (39) , in USA by Gary, et al. 2003 (40)

The prevalence of HBV infection among pregnant women in relation with history of blood transfusion

The blood and blood products play a good rule as a mode of transfusion of hepatitis B&C infection. In this study, there is a significant statistical association between previous blood transfusion and HBcAb (IgG) $p < 0.05$ but there is non significant association between blood transfusion and HBsAg ($p > 0.05$).

Studies done by Blankson, et al.2005 in Ghana(50), Adwuji, et al.1996 in Nigeria(51) & Tess, et al.2000 in Tanzania(52) found that a history of blood transfusion was not always significant among HBsAg carriers.

The prevalence of HBV infection among pregnant women by history of intravenous injecting

The highest prevalence of HBV infection is found among those who have a history of intravenous

injecting (significant statistical association). This may be due to exposure to contaminated needles or syringe. This finding is in agreement with those recorded in Saudi Arabia by AL-Kuwari, et al. 2007[53], in Pakistan by Khan, et al.2000[54] & in China by Ting, et al.2006[55].

The prevalence of HBV infection among pregnant women in relation with history of vaccination

In the present study, there is significant statistical association observe between non vaccination with HBV vaccine and prevalence of HBV infection. Similar findings was detected between the unvaccination personal & HBV infection in USA by Charles, et al. 1999[56] and in Palestine by Rola, et al. 2005 [57]. Our findings indicate that vaccination act as a good way to prevent infection with HBV infection.

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Detection Of The Local Isolates Of *Burkholderia cepacia* and their Virulence Factors

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ABSTRACT

This study was carried on seven clinical isolates of B. cepacia to detect some virulence factors (hemolysin,urease ,protease ,gelatinase ,lipase, sidrophore production & exotoxic complex) .
The results showed that all the isolates were capable to produce protease & sidrophore ,while71.5% of these isolates produce urease ,28.5% produce hemolysin & non was capable to produce lipase .Exotoxic complex (ETC) detection experiments were carried on B. cepacia S3 & results showed that 300µl of the crude (ETC) cause death of the laboratory mice .

Key Words: *Burkholderia cepacia* ,Hemolysin,Protease,Lipase,Urease,Exotoxic complex& Sidrophore

INTRODUCTION

B. cepacia Infections are always associated with cystic fibrosis disease [1]& immunocopromised patients which received a special care [2] .This bacterium was considered as a opportunistic microorganism & a causative agent of sepsis ,contaminated burns &,surgical wound & pericarditis [3,4,5] .*B.cepacia* produces virulence factors that enable it to spread to blood stream [6] ,one of these virulence factors is sidrophore which strongly binds iron & introduce it to the bacterial cells [7,8,9] . This bacterium has the ability to produce extracellular enzymes like protease that digests collagen & gelatin [10,11],lipase, phospholipase & urease [12,13]

.Other studies mentioned that this bacteria is capable to produce extracellular toxins ,named as exotoxic complex (ETC) ,which cause pathogenicity & death of human .Infection with *B.cepacia* are associated with fever ,damage of respiratory system cells & hypercytopenia [14] .

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MATERIALS AND METHODS

Bacterial strains : seven clinical isolates of *B.cepacia* [15] & a standard strain of *Pseudomonas aeruginosa*(ATCC 27853) as a control .

Laboratory animals: mice (weigh 20 gm) .

Detection of virulence factors:

Hemolysin ,urease ,gelatinase,lipase, & Tween-80 tests were carried according to [16,17] .Sidrophore production test was carried out as described by [18] .

Production of exotoxic complex :

Production medium was prepared according to [19], inoculated with *B. cepacia* S3 & incubated at 37C◦ for 72 h. (shaking speed 150 rpm/min.).Bacterial cells were precipitated by centrifugation (8000 rpm/min.) for 20 min. The supernatant was filtrated by ultrafiltration & concentrated with glucose & dialyzed with Tris-HCl buffer at 4C◦ for 12 h. ,buffer was changed twice. .The dialyzed solution was sterilized using (.22 µ) Millipore filters &stored at (-20C◦) .

Characterization of (ETC) :

Thermal treatment : 1 ml of ETC extract was incubated at 100C◦ for 15 min. & then injected in a laboratory mice .

Chemical treatment: 100 µl of 10N NaOH was added to 900µl of ETC extract & incubated at 66C◦ for 18 h. . pH of the solution was neutralized by addition of 10N HCl & injected in a laboratory mice .

Injected laboratory mice monitored for 2 days & both results were recorded .

Determination of lethal dose of (ETC): four aliquots of ETC (100,200,300,& 400µl)were injected intraperitoneal in a laboratory mice. The same volumes of dialyzed growth

medium were also injected into laboratory mice as control.

RESULTS

The results in table (1) showed that only five isolates were urease positive ,& all isolates produced protease & melted the gelatin agar within 48 h.
Isolates were able to lyse milk protein & produce translucent zone around bacterial growth within 48 h., on the egg-yolk agar medium ,a pearly zone was produced as a result of *B. cepacia* growth .All the isolates were lipase negative. Only two of the isolates were capable to produce hemolysin on blood agar medium ,while all isolates were able to grow on sidrophore production medium better than the standard strain *Pseudomonas aeruginosa* . The lethal dose of(ETC) for mice was 300µl ,& the crude (ETC)was heat stable & its lethal effect was inhibited by NaOH

TABLE (1): :Virulence factors produced by B.cepacia

Isolate	Gelatinae	Hemolysin	Lipase	Phospholipase(mm)*	Protease(mm)*	sidrophore	Urease
S1	+	-	-	10	12	+	+
S2	+	+	-	10	14	+	+
S3	+	+	-	9	8.5	+	-
S4	+	-	-	10	10	+	+
S5	+	-	-	9	12	+	-
S6	+	-	-	10	12	+	+
S7	+	-	-	12	12	+	+

*Diameter of zone

DISCUSSION

As shown in table (1) ,five isolates out of seven were urease positive ,so this result indicate the ability of bacteria to cause urinary tract infections ,because urease converts urea to ammonia which binds to mucous & enables bacteria to colonize [21] .*B. cepacia* can be considered as a virulent opportunistic bacteria because of its ability to produce many

virulence factors ,like protease ,gelatinase & phospholipase [22] .Five of the isolates were unable to produce hemolysin ,& this result could be referred to the presence of cholesterol in blood cell which inhibits blood cells lysis . [23] .

B. cepacia S3 was selected as (ETC) producer ,because of its low productivity of proteolysis enzymes ,& this property helps in avoiding interference of protease toxic effect with (ETC) & preventing the lytic effect of protease on (ETC).Our results showed that 300μl of (ETC) was enough to kill the experimental mice ,& these results were supported by results obtained previously [14].*B. cepacia* S3 is efficient in production of (ETC) .Thermal treatment of (ETC) revealed that heat has no effect on its activity ,so we can conclude that the protein portion is not responsible fo toxicity of (ETC) while the chemical treatment with NaOH inhibits its activity , by this result LPS may responsible of toxicity of (ETC) [20]. Some results from other studies mentioned that there is no role of cell wall LPS in the toxicity ,because (ETC) was determined in the extract. Other studies mentioned the absence of high toxicity of cell wall or bacterial lysate injected in mice [14].

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الزيادة الحاصلة في الأكسدة الفوقية للدهون وعلاقتهـــا بفيتـــامين أ والسيلينيوم نتيجة التلوث بالرصاص .

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ABSTRACT

Discussed the increase of the chemical evidence of life to the process of oxidative meta-fat (MDA) through the mechanical damage of free radicals have been linked to tissue factor exposure to lead.

Were selected (40) of workers exposed to lead through their daily work and divided into two groups according to duration of exposure was selected as the control group (10) of healthy volunteers to lead exposure of others.

Measured by the proportion of lead and bilateral (MDA), as well as Vit-A, and selenium, where the totals of these results showed a marked increase in the proportion of lead, (MDA) in the proportion of Onqassan and Vit-A, and selenium in the blood serum of workers and duration of exposure as compared with the control group.

Results were discussed in the light of modern scientific theories that explain these products Shetty Metadata fat.

Method is used (Fong) to measure the neutral level (MDA) was estimated as a shot using the atomic absorption of Others WAHBI (Ass-GF)

الملخص باللغة العربية

تناول البحث زيادة الدليل الكيميائي الحياتي لعملية الأكسدة الفوقية للدهون (المـــالون ثنـــائي الالديهايـــد MDA) مـــن خـــلال ميكانيكية الجذور الحرة على تلف الأنسجة وتم ربط هذا العامل بالتعرض للرصاص .

تم اختيار (40) من العاملين المتعرضين للرصاص من خـــلال عملهـــم اليـــومي وقسموا إلـــى مجمـــوعتين حـــسب فتـــرة

التعرض كما اختير كمجموعة سيطرة (10) من المتطوعين الأصحاء الغير متعرضين للرصاص .

قيست نسبة الرصاص والمالون ثنائي الالديهايد(MDA), وكذلك Vit-A, والسيلينيوم لهذه المجاميع حيث أظهرت النتائج زيادة ملحوظة في نسبة الرصاص و (MDA) ونقصان في نسبة Vit-A, والسيلينيوم في مصل دم العاملين وحسب فترة التعرض مقارنة مع مجموعة السيطرة .

نوقشت النتائج على ضوء النظريات العلمية الحديثة والتي تفسر تكون هذه النواتج الثانوية للأكسدة الفوقية للدهون .

استخدمت طريقة (Fong) اللونية لقياس مستوى (MDA) كما تم تقدير عنصر الرصاص باستخدام جهاز الامتصاص الذري الغير لهبي (Ass-G.F)

المقدمة

يعتقد بان الرصاص من العناصر غير الضرورية للكائنات الحية ولاسيما اللبائن [(1)] ولقد أظهرت الدراسات أن الأعضاء الرئيسية التي تكون هدفا لتأثير الرصاص هي الأنسجة الرخوة مثل القناة المعدية – المعوية (Gastro intestinal) والرئة والكبد . كما يمكن أن يؤثر الرصاص في حالات التعرض المزمن في الجهاز العصبي والجهاز الوعائي – القلبي Gardio-vascular system والجهاز المناعي وجهاز تخليق الهيم Heme biosyuthesis system فضلا عن الكلى والجهاز التناسلي [(12-10)] .

يسبب الرصاص تأثيرات مرضية للرئة وخاصة عند التعرض لدخان أو غبار ملوث بمركباته فقد أظهرت إحدى الدراسات أن التعرض للرصاص يؤدي إلى حدوث تلف بأكثر من 90% من الخلايا البلعمية Macrophagesفي الحويصلات الرئوية في خنازير غينيا بعد مرور 20 ساعة من فترة التعرض [(19)] .

أن التلوث بمركبات الرصاص اللاعضوي ينتج من استنشاق كميات كبيرة من أكسيد الرصاص خلال مدة العمل من قبل العاملين في معامل البطاريات وحقل الفخار والمطابع وصناعة الأصباغ وأحيانا في أوعية حفظ الأطعمة وكمادة مانعة لفرقعة احتراق البنزين في مكائن الاحتراق الداخلي حيث يضاف بشكل مركب رابع اثيل الرصاص [(5)]

فالرصاص فلز ثقيل ويعد من الفلزات الثابتة في الهواء الجاف أما عند وجود الرطوبة في الجو فانه سرعان ما يكون

أحادي أكسيد الرصاص ثم يكون كاربونات الرصاص مع ثاني أكسيد الكربون [1,2,3].
ان وجود الرصاص كفلز فانه يوجد على شكل مركبات عضوية أو لا عضوية وانه الشكل الأكثر شيوعا حيث تبلغ نسبة الأملاح اللاعضوية للنحاس اكثر 95% من الرصاص الكلي في البيئة [4] .

طريقة العمل :

1- طريقة تقدير مستوى الأكسدة في مصل الدم بمقدار ما يتكون من MDA ان المالون ثنائي الالديهايد هو من النواتج الثانوية للأكسدة الفوقية للدهون فان قياس هذه المادة يعطي انطباعا عن مستوى الأكسدة واستخدمت طريقة لونية تعتمد على التفاعل بين مركب حامض الثايو باربتيورك والمالون ثنائي الالديهايد ليغطي مركبا لونياعلى امتصاصية له في 532نانومتر(5) واستخدمت المحاليل التالية

أ- 0.5%(وزن-حجم)ثلاثي كلورو حامض الخليك(TCA)

ب- 0 .5% (وزن-حجم) حامض الثايو بارتيك (TBA)

ج- 70% ثلاثي كلورو حامض الخليك TCA

د-كلورو فورم

2-قياس كمية فيتامين A في مصل الدم بتقنية ال HPLCباستخدام الطور المتحرك(% 99)ميثانول (1%) ماء مقطر بطول موجي330 nm

نوع العمود (ODS) C-18 [16].

3- قياس عنصر السلينيوم

حضرت محاليل قياسية مختلفة للعنصر Se لغرض عمل منحني قياسي للعنصر وذلك بسبب كميات مختلفة من العنصر في قناني حجميه وإكمالها للعلامة بالماء الأيوني.

النتائج والمناقشة

نلاحظ من الجدول رقم (1) الزيادة في معدل مستوى الرصاص لمجاميع العمال مقارنة بمجموعة السيطرة وان هذه الزيادة تتناسب طرديا مع طول الفترة الزمنية تتعرض لملوثات الرصاص .

كذلك نلاحظ من جدول رقم (2) الزيادة في معدل مستويات الأكسدة الفوقية للدهون لمجاميع العمال مقارنة بمجموعة السيطرة حيث نلحظ هذه الزيادة مع الفترة الزمنية للتعرض . ويبين جدول رقم (3) انخفاض

في مستوى تركيز فيتامين (A) بالنسبة إلى العاملين مقارنة مع قيم الـــسيطرة ونجـــد انخفاضا في مستوى فيتامين (A) مع طول مـــــــدة التعـــــرض .

جدول (1)

بين معدل مستوى الدلالة ال (pb) عند مجموعه العاملين

T	Mean	(µg/dL) pb معدل الـــ ±S.D	العدد	المجاميع
---	20.1	7.6	10	Control C
P<0.05	149.22	9.1	20	A
P<0.001	200.24	21.3	20	B

A = مجموعة العمال (من سنة إلى 10 سنوات).

B = مجموعة العمال (من 11 سنة فما فوق).

C = مجموعة السيطرة

جدول رقم (2)

يحدد معدل الانحراف القياسي والمعدل ومستوى الدلالة (MDA)

عند مجموعة السيطرة ومجاميع العمال.

T	Mean	(n mol/dL) (MDA) معدل ±S.D	العدد	المجاميع
---	26.5	5.6	10	Control C
P<0.05	86.8	3.1	20	A
P<0.001	120.2	8.2	20	B

A,B,Cكما في جدول رقم (1)

جدول رقم (3)

يحدد معدل الانحراف القياسي والمعدل ومستوى الدلالة لفيتامين

Aعند مجموعة السيطرة ومجاميع العمال.

المجاميع	العدد	(mg/dL) (Vit-A) معدل ±S.D	Mean	T
Control C	10	10.2	69.8	---
A	20	14.2	44.6	**P<0.05**
B	20	16.7	35.05	P<0.001

A,B,Cكما في جدول رقم (1)

يعد فيتامين A من مركبات الكاروتينات التي لها فعالية كمضادة للأكسدة (118,119) وان فعاليته الكيميائية تأتي من السلسلة الطويلة الحاوية على أواصر مزدوجة متعاقبة التي تكون معوضة بمختلف المجاميع أن ال Ros التي يمكن أن يكتسبها فيتامين A هي O_2 وجذر البيروكسي (peroxy radical) (12)

ولفيتامين A عدة وظائف منها المشاركة في عملية الإبصار بالتفاعل مع بروتين (obsin) مكونا الأرجوان البصري كما يعد ضروريا في تكوين الكاربوهيدرات المخاطية المكونة لمادة مخاطية لإفراز الطبقة الطلائية التي توفر الحماية للقنوات الجسمية مثل القنوات التنفسية والبولية والتناسلية وكذلك ملتحمة العين والقرنية واللثة.

كذلك للفيتامين دور في تكوين عدد من الهرمونات مثل الكورتزون(cortisone) والذي تفرزه غدة الأدرنالين والتي لها دور في تمثيل الدهون والكاربوهيدرات و(vit a.) أهمية في تقليل مستوى الأكسدة ويعد من الفيتامينات المضادة للأكسدة وكما هو معلوم فان بيتا- كارتوين(B-carotene) يعتبر المولد للفيتامين(A) وهو أيضا من المواد المضادة للأكسدة ويوجد بيتا – كاروتين في(LDLC) مع فيتامين(E) وبذلك يمنع أكسدة (LDLC) .

كما يوصي بتناول فيتامين(A) لمـا لـه أهمية في الحفظ على شبكة العـين ومـا تحميه العين والقرنية من الأضرار التـي تلحق بالعين وكذلك لفيتامين (A)دور في رفع مستوى(apo-A) ومـن ثـم رفـع (HDLC) في الدم [16].

إذ استطاع البـاحثون تفـسير ميكانيكيـة الزيادة في معدل الأكسدة الفوقيـة بـسبب ارتفاع مستويات (Ros)وهـي مولـدات للجذور الحرة وسوف يحصل زيادة فـي التلف الحاصل في الخلايا ويـسرع مـن عملية انتقال الالكترونات والأكسدة الفوقية للدهون في الأنسجة البيولوجية داخل الخلية الحية وان هذه النتائج تتفق مـع بحـوث أخرى وعند زيادة هذه الأكسدة فان الجذور الحرة المتولدة والمتزايدة تودي إلى التلف لكثير من الأغـشية الخلويـة والنهايـات العصبية في الدماغ[17-18].

تبين من جدول رقم (4) انخفاض مستوى الـسيلينيوم مـع زيـادة مـدة التعـرض للرصاص ويعلم دورهما زيادة اسـتهلاك السلينيوم الذي يلعب دورا مهمـا وكمـادة أساس فـي عمـل إنـزيم الكلوتاثـايون بيروكسيديز حيث يعتبر هذا الإنزيم مـن الإنزيمات المضادة للأكسدة ويتواجد فـي معظم أعضاء الجسم .

جدول رقم (4)

يحدد معدل الانحراف القياسي والمعدل ومستوى دلالة تركيز السيلينيوم

عند مجموعة السيطرة ومجاميع العمال.

T	Mean	(µg/dL) Se معدل الـ ±S.D	العدد	المجاميع
---	9.2	0.92	10	Control C
P<0.01	8.3	0.81	20	A
P<0.001	6.1	0.72	20	B

وهناك أنواعا مختلفة من أنـــزيم الكلوتـــا ثايون بيروكسديز التي تعتمد على عنصر السلينيوم في عملها . وظيفة هذه الإنزيمات كمضادات للأكسدة هي الاحتفاظ بمستويات مضادات الأكسدة الداخلية مثل مـــستويات الكلوتاثـــايون المختـــزل (GSH) الـــذي تحصل عليه من اختزال الكلوتـــا ثـــايون المؤكسد (GSSG) بفعل إنـــزيم الكلوتـــا ثايون,هيدكنيز (Glutathion 1.6.4.1 (10)reductase) Ec

كما يعتبر الـــسلينيوم ضـــروري لنظـــام المناعة والغدة الدرقية [11] .

إن من أهم وظائف هذا المعدن وأكثرهـــا شهرة دوره كمضاد للأكسدة وللـــسرطان [12]

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دراسة تأثير فيتامين E و تركيز بعض العناصر الأساسية والحيوية للجسم لمرضى داء السكري

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ABSTRACT

In this research was to study the impact of some of the basic elements and vitality of the body, and the relationship of these elements and vitamin E for patients with diabetes, patients were divided on the different periods of time, into two groups, in addition to a third control group of non-pation.

Include the first set (20 a) for a period of time 5 to 20 years old) The second group also includes (20 patients.) for a period of time (20 years And over). The control group included (20 people) of the non-patients.

The study involved measuring the level of vitamin E in patients in addition to the elements selenium. Iron, magnesium,. And found there was a rise in the level of magnesium, iron, compared with the control group with a different time period of the patients.

decline in the level of selenium and vitamin E compared with the control group with a different time period for patient

الملخص

تم في هذا البحث دراسة تأثير بعض العناصر الأساسية والحيوية للجسم وعلاقة هذه العناصر و فيتامينE لمرضى داء السكري، تم تقسيم المرضى على فترات زمنية مختلفة, إلى مجموعتين بالإضافة إلى مجموعة ثالثه هي مجموعة السيطرة من غير المرضى .

تشمل المجموعة الأولى (20 مريض) لفترة زمنية من (5 إلى 20 سنه)أما المجموعة الثانية تشمل أيضا (20 مريض)

لفترة زمنية من (20 سنة فما فوق). وكانت مجموعة السيطرة تشتمل على(20 شخص) من غير المرضى .
لقد شملت الدراسة قياس مستوى فيتامينE لدى المرضى بالإضافة إلى عناصر السيلينيوم. الحديد , والمغنسيوم ,.فوجد هنالك ارتفاع في مستوى المغنيسيوم , والحديد مقارنة مع مجموعة السيطرة مع اختلاف الفترة الزمنية للمرضى . كما لوحظ انخفاض في مستوى السيلينيوم وفيتامين E مقارنة مع مجموعة السيطرة مع اختلاف الفترة الزمنية للمرضى .

مفاتيح دالة : مرض داء السكري ، فيتامين E. الحديد ،السيلينيوم ، المغنيسيوم

المقدمة

السُكّري أو الداء السكري أو المرض السكري وغيرها، يطلق عليه باللاتينية (Diabetes mellitus) هو متلازمة تتصف باضطراب وارتفاع شاذ في تركيز سكر الدم الناجم عن نقص الأنسولين ، أو انخفاض حساسية الأنسجة للأنسولين، أو كلا الأمرين[1].
يتصف مرض السكري بارتفاع سكر الدم (الجلوكوز)عن المستوى الطبيعي مما ينتج عنه طرح الجلوكوز في البول فيؤدي إلى كثرة التبول والشعور بالعطش . يحدث مرض السكر نتيجة نقص أو عدم فعالية هرمون الأنسولين الذي تفرزه غدة البنكرياس وغالبا ما يكون مزمنا ، وقد يكون وراثيا أو مكتسبا عند وجود العوامل المساعدة على الإصابة به ، من العوامل المساعدة للإصابة بداء السكري : السمنة أو البدانة .الوراثة .المناعة ،الحمل ، الالتهابات ،الأدوية الهرمونات ،أمراض البنكرياس ، الصدمة أو الخوف .
وقد أظهرت البحوث الحديثة أن عنصر المغنيسيوم يتمتع بخصائص وقائية قوية ضد مرض السكري، فيقلل من خطر الإصابة به من خلال تأثيره على هرمون الأنسولين وزيادة فعاليته في تنظيم مستويات السكر في الدم حيث .يلعب المغنيسوم دورا مهما في العديد من وظائف الجسم الحيوية إذ يدخل في عمليات تمثيل وإطلاق الطاقة في الجسم، وفي عمليات بناء البروتين، وتنظيم درجة حرارة الجسم، وتقلص العضلات، وتناقل السيلانات العصبية، وصحة وتثبيت العظام، وفي الحفاظ على ضغط الدم. ويدخل عنصر المغنبيسيوم في أكثر من 300 تفاعل من تفاعلات الجسم، وبالأخص تلك المتعلقة بتمثيل الأحماض الدهنية الأساسية، وبناء البروتين، ويرتبط

بالألبومين بنحو 22% منــه 7% يــرتبط بالكلوبيولينات أما النسبة الباقية التي تبلغ 71% تقريبا من المغنسيوم كعامل مساعد في عدد من الأنزيمات التي تستخدم مركب ال ATP كمادة أساس يوجد فــي جميــع الأنسجة والعظام وان نسبة توزيعه تكــون متساوية بين الأنسجة الرخــوة والعظــام .[2]

ومن المعادن الأخرى الضرورية في جسم الإنسان هو سيلينيوم. ,وهو جزءُ مهمُ مِنْ إنزيماتِ مانع التأكسد الذي يَحْمي الخلايــا ضدّ تأثيراتِ الجذور الحرّةِ التي تُنتجُ أثناء أيضِ أوكسجينِ. هنالك أنواعاً مختلفة من أنزيم الكلوتاثايون بيروكسيديز التي تعتمد على عنصر السلينيوم في عملها. وظيفــة هذه الأنزيمات كمضادات للأكــسدة هــي الاحتفاظ بمستويات مــضادات الأكــسدة الداخليــة مثــل مــستويات الكلوتاثــايون المختزل (GSH) الذي تحصل عليه مــن اختزال الكلوتاثايون المؤكــسد (GSSG) بفعل أنزيم كلوتاثــايون ريــدكتييز (EC 1.6.4.1) (Glutathion reductase) [3]

وكذالك يعتبر السيلينيوم ضروريُ لنظــام المناعة والغدّةِ الدرقيةِ[4] ،إن مــن أهــم وظائف هذا المعدن وأكثرها شــهرة دوره كمضاد للأكسدة وللسرطان [5].من أكثــر المعادن الانتقالية في الأنظمة البيولوجيــة الحديد وهو أساس لمعظم الكائنات الحيــة ويسهم في عمليات حيويــة كثيــرة مثــل ميكانيكيات الأكسدة الحيوية فــي الخليــة وكذلك نقل الأوكسجين إلى الأنــسجة [6] حيث يمثل الحديد العنصر الأســاس فــي هيموغلوبين الــدم والمــايوكلوبين وفــي أنزيمات كثيرة مثل

Cytochrom Oxidase, Catalase, Superoxide, Dismutase, Peroxidase, ويمتص الحديد في الأمعاء وينقل بوساطة البروتين الناقل الموجود في البلازما (Trasferrin) إلى أماكن تخزينه في نخــاع العظــم حيــث يحــوى فــي الهيموغلوبين وكذلك ينقــل إلــى الكبــد والطحال. إن الحديــد المخــزون يــشكل (Ferritin) تحصل فيه عملية توازن تنظم بشكل أولي عن طريق الامتصاص وليس عن طريق الطرح أن مستويات الحديد في البلازما تتراوح بين (60-150 μg / dL) [7]تؤثر زيادة الحديد في بعض الأعــضاء الداخلية في الجسم مثل غــدة البنكريــاس وعلى الخلايا التي تفرز هرمون الأنسولين (جزر لانجرهانس) مما يؤدي إلى تلفهــا وفشلها في إفراز هذا الهرمــون والــذي يؤدي إلى ظهــور داء الــسكري يعمــل فيتامين (E)في السيطرة علــى مــرض

السكر حيث يخفض إلى حد كبير الضرر الوعائي المدمر الذي يرافق مرض السكر [8].

طرائق العمل

أولا : قياس فيتامين(E,) في مصل الدم بواسطة تقنية كروماتوغرافيا السائل *عالي الأداء* HPLC ، تم قياس فيتامين E باستخدام الطريقة المحورة من (Deleen heer) حيث تم تحضير محلول مانع الأكسدة والمحلول القياسي باستخدام طور متحرك (99%) ميثانول (1 %) ماء مقطر بطول موجي 287nm , نوع العمود (ODS) C-18 [9].

ثانيا: حضرت محاليل قياسية مختلفة للعناصر(Mg,Fe,Se) لغرض بناء منحني قياسي لكل عنصر من العناصر أعلاه ، وذالك بسحب كميات مختلفة من كل عنصر في قناني حجمية وإكمالها للعلامة بالماء الأيوني، ومن ثم يتم قياس كل مجموعة محاليل القياسية الخاصة لكل عنصر باستخدام مطيافيه الامتصاص الذري, فبالنسبة للعنصرSe [10,11]. تم استعمال المطياف الذري غير اللهبي ويكون مقدار الحجم الذي يدخل للجهاز هو (10µL) من كل محلول قياسي تم تحضيره أما العناصرMg ,Fe, فيتم قياس باستعمال المطياف الذري اللهبي ويكون الحجم الداخل إلى الجهاز هو (0.8mL)لكل محلول قياسي وبعد حصولنا على المنحني القياسي لكل عنصر يتم قياس عينات الدم باستخدام (فنل خاص) بحقنة مقدارها (0.25mL) من النموذج . تؤخذ القراءة بجهاز المطياف الذري اللهبي . الجهاز المطياف الذري غير اللهبي فانه يتم سحب (10µL) لكل نموذج , ويتم القياس باستخدام الأطوال الموجية الرئيسة للعناصر أعلاه.

النتائج والمناقشة

يبين لنا الجدول رقم (1) النقص الحاصل في معدل فيتامين (Vit-E) هو أحد مضادات الأكسدة الذائبة في الدهون ويعمل على حماية الدهون الموجودة في الأغشية الخلوية من التلف التأكسدي وكذلك فان فيتامين (E) له تأثير على الاستجابة المناعية الخلوية (Cellular Immune Response) حيث يعمل على تقوية الحالة المناعية[12] أن فيتامين E له أهمية كبيرة وهو الأفضل من مضادات الأكسدة الذائبة في الدهون والأهمية العظمى لعمله هي في تثبيط الأكسدة الفوقية وكسح جذر دهن

البيروكسيد ليعطي الهيدروبيروكسيد الدهني وجذر التوكوبيروكسيد والأخير هو اقل فعالية من جذر البيروكسيد تجاه الحوامض الشحمية غير المشبعة المجاورة وبهذا يعمل كمضاد للأكسدة عن طريق كسر سلسلة الأكسدة [13]، وعند مقارنة فيتامين (E) بباقي مضادات الأكسدة الكارهة للماء فانه أكثر تأثير في حماية الخلية من الأكسدة الفوقية للدهون وإن القلة الملحوظة في (Vit-E) نتيجة التلف الحاصل فيه وتقليل الاستجابة المناعي [14]للعاملين كما إن تأثير فيتامين (E) يمثل في تحفيز الخلايا اللمفاوية التائيه المساعدة (T. Helper Lymphocytes) والتي تعمل على تحويل إنتاج الأجسام المضادة من نوع (IgM إلى نوع IgG) [15]إن النتائج التي توصلنا إليها في بحثنا هذا تتفق مع ما جاء في نتائج الباحثين . [16] فيتامين (E) فإنه مضاد للأكسدة، وقد يفيد في تقليل مضاعفات السكر، بعض الأبحاث تدل على أن تناول جرعات من فيتامين E يساعد على الوقاية من انسداد شرايين القلب التاجية وربما كان ذلك مفيداً لمرضى السكري.

جدول رقم (1)

يحدد معدل الانحراف القياسي والمعدل ومستوى الدلالة لتركيز فيتامين (E) عند مجموعة السيطرة ومجاميع المرضى.

T	Mean	±S.D معدل (Vit-E) (mg/dL)	العدد	المجاميع
---	1.24	0.16	20	Control C
P<0.05	0.910	0.20	20	A
P<0.001	0.812	0.24	20	B

Aمجموعة المرضى (5 الى 20 سنة) .

B = مجموعة المرضى (من 20 سنة فما فوق).

C = مجموعة السيطرة

يبين جدول رقم (2) أن معـدل مـستوى المغنسيوم يزداد زيـادة واضـحة لـدى مجاميع المرضى مقارنة مـع مجموعـة السيطرة، وان هناك علاقة طرديـة بـين مـستوى إنـزيم ال SOD وعنـصر المغنسيوم في مـصل دم المرضـى . إن وجود المغنسيوم في السوائل داخل الخليـة يكون بتركيز عالي جدا عنه في الـسوائل خارج الخلية ويمكن أن يعزى سبب الزيادة في التراكم خـارج الخليـة إلـى التلـف الحاصل للخلايا ومن زيـادة نـسبته فـي مصل الدم . للمغنيسيوم دور هام في إنتاج الطاقة بالجـسم وتجديـد الخلايـا ونقـل الإشارات العصبية بالإضافة إلـى كونـه منشط لبعض إنزيمات الخلايا. ربما تكون هناك حاجة لإضافة المغنيـسيوم لـبعض مرضى السكري الفاقدين للـسيطرة علـى نسبة السكر بالدم حيث أن نقـصه يـؤدي لنقص فعالية الأنسولين وبالتـالي ارتفـاع نسبة السكر بالدم.

جدول رقم (2)

يحدد معدل الانحراف القياسي والمعدل ومستوى الدلالة للمغنيسيوم

المجاميع	العدد	S.D± معـدل الـ Mg (μg/dL)	Mean	T
ControC	20	103.28	1690.70	--
A	20	602.6	2050.18	P<0.001
B	20	843.9	2532.62	P<0.001

C,B,A كما في جدول رقم 1

يبين جدول رقم (3)انخفاض في مـستوي السيلينيوم مع طول الفترة الزمنية للمرض ويعلل دوراهما زيادة استهلاك الـسلينيوم الذي يلعب دورا مهما وكمادة أساس فـي عمل إنزيم الكلوتاثايون بيروكسيديز حيث يعتبر إنزيم الكلوتاثايون بيروكسيديز مـن الإنزيمات المضادة للأكسدة ويتواجد فـي معظم أعضاء الجسم.

جدول رقم (3)

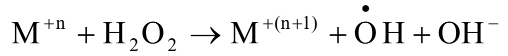
يحدد معدل الانحراف القياسي والمعدل ومستوى الدلالة للسيلينيوم

T	Mean	Se معـــــدل الــــــ ±S.D (µg/dL)	العدد	المجاميع
--	0.93	0.14	20	Control C
P<0.001	0.82	0.94	20	A
P<0.001	0.75	0.36	20	B

C,B,A=كما في جدول رقم 1

يبين لنا الجدول رقم (4) إن مستوى الحديد الحر في المصل يزداد في مجاميع المرضى مقارنة بمجموعة السيطرة. ويمكن تفسير ذلك بان الحديد محفزاً للأكسدة الفوقية للدهون من خلال تفاعل (فنتون).

إن أيونات الحديد تعمل على اختزال (H_2O_2) من خلال تفاعل فنتون وهي بذلك تولد نوعاً من أنواع الـ (ROS) التي تكون اخطر من (H_2O_2) نفسه مثل جذر ($O^{\bullet}H$) داخل الجسم (Invivo) حيث إن تفاعل فنتون يتبع المعادلة التالية: –



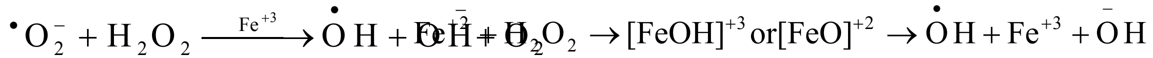
$$M^{+n} + H_2O_2 \rightarrow M^{+(n+1)} + \dot{O}H + OH^-$$

وإن (M^{+n}) حديد أو نحاس.[17]

إن التفاعل الذي يقوم به الحديد معروف ويمكن تمثيله بالمعادلة والميكانيكيات المقترحة

$$Fe^{+2} + H_2O_2 \qquad Fe^{+3} + O^{\bullet}H + OH^-$$

إن الناتج الأولي لتفاعل فنتون قد يكون معقد حديد أوكسجين (Oxo-iron complex) باحتمال تكون الفيريل (Ferryl) والذي يتحلل ليعطي جذر الهايدروكسيل وفق المعادلة التالية.



إن تكون جذر الفيريل الـذي فيـه يكون العد التاكسدي للحديد (4+) قد اقترح من قبل العالم (Walling, C) [18]، كما إن جذر الفيريل (Ferryl radical) يكون جـذراً فعـالاً فـي بعـض أنزيمـات البيروكسيديز.

أما التفاعل بين جذر ($^{\bullet}O^{-}_{2}$) والـ (H_2O_2) بوجود أيونات الحديديك (Fe^{+3}) قد طلق عليه تفاعل هابر- ويبس المحفـز بالحديـد (Iron-Catalyzed Haber (Weiss reactions

الذي يطلق عليه في بعض الأحيان السوبر اوكسايد المؤدي إلى تفاعل فنتون

(Super Oxide driven Fenton Reaction)

إن هذا التفاعل يؤدي إلى جزء مـن التلف الحاصل في الخلايا الحية وذلك عن طريـق تولـد الـــ (ROS). إن الـشد التاكـسدي (Oxidative Stress) يعمـل على تحرير الأيونـات مـن البروتينـات المرتبطة بها وبهذا تجهز هـذه الأيونـات لتفاعلات الجذور الحرة ممثلاً (O_2) يمكن أن يحرر الحديد من الفرتين (Ferrittin) لذا سوف يرتفع مستوى الحديد في المصل [19] ، عند النتائج الأولية تـستدل علـى وجود تلف جزئ حاصل في الخلايا الحية وذالك يؤدي إلى تحريـر الايونـات مـن البروتينات المرتبطة بها

جدول رقم (4)

يحدد معدل الانحراف القياسي والمعدل ومستوى الدلالة للـحديد

T	Mean	Fe معـــدل الــــ ±S.D (µg/dL)	العدد	المجاميع
---	193.9	14.25	20	Control C
P<0.01	253.3	40.31	20	A
P<0.01	341.5	51.12	20	B

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دراسة خواص لحام سبيكة (كوبلت _كروم) باستخدام تقنية الليزر

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الملخص

إن الهدف من هذه دراسة خواص السبيكة الملحومة قبل وبعد معاملتها بأشعة الليزر ،وبيان نوعية المواصفات المعتمدة في تقييم السبيكة الملحومة تم إجراء اللحام(نوع التوصيل المحدود)، باستخدام ليزر النيديميوم- ياك لسبيكة (كوبلت_ كروم) المستخدمة في طب الأسنان, فتضمن البحث دراسة مواصفات منطقة اللحام والتي منها تم قياس عرض المنطقة المتأثرة ،عمق اللحام ،قطر التفاعل مع الطاقة.بالإضافة لفحوصات الميكانيكية كالشد والصلادة من خلال النتائج أن استخدام تقنية الليزر في اللحام يكون سببا في الحصول على مواصفات أفضل من ما هو عليه في حالة استخدام الطريقة التقليدية وذالك بسبب فاعلية الليزر في التسخين السريع لإحداث التفاعلات المطلوبة، وقد امتلكت منطقة اللحام مقاومة شد عاليه مقارنة بالطريقة التقليدية ،وكذلك اظهر الفحص المجهري تحسين نعومة السطوح انعكاسا لتجانس السبيكة ، وخاليا من الشروخ بعد معاملتها بتقنية الليزر .

<u>المفاتيح الدالة :</u>

لحام الليزر . سبيكة كوبلت كروم ،التركيب المجهري . الصلادة . اختبار الشد

المقدمة

في اللحام اخذ الليزر مكانة لا يستهان بها من حيث الاستخدام بالمقارنة مع باقي المصادر الأخرى كالقوس الكهربائي والحزمة الالكترونية ، وذلك بسبب الإمكانيات الكبيرة التي تتميز بها ليزرات النيديميوم وثاني أكسيد الكربون ،تحققت أول عملية لحام في عام 1963وذلك بواسطة ليزر الياقوت (Ruby laser) وكذلك تمكن (Anderson $ Jachson)عام 1965من إجراء عملية لحام على النحاس والنيكل [1]. بعد ذلك استمرت الأبحاث بهذا المجال حيث بدأت عملية اللحام في التقدم بخطوات سريعة جدا مع تطور التكنولوجيا الحديثة

خلال النصف الأول من القرن العشرين ومعه تتابع ظهور الأساليب المختلفة للحام المعادن وسبائكه، ففي نهاية الثمانينات قدمت عملية اللحام باستعمال الليزر دفعة كبيرة في مجال طب الأسنان وذلك بتطوير الأجهزةِ الأرخصِ ثمنا والأصغرِ حجما وذلك للامتلاك شعاع الليزر الخاصية التي تجعله يدخل في تشكيلة واسعة من التطبيقات العلمية والصناعية والطبية[2,3]. ومن الليزرات التي تستخدم في التطبيقات المختلفة وخاصة علم المواد هو ليزرات (الحالة الصلبة) ,وان لكل نوع من هذه الليزرات مواصفاته الخاصة به التي تحدد مجالات استخدامه .استخدم ليزر النيديميوم- زجاج في اللحام والتصلب السطحي لطاقة نبضِته عاليةِ ويعود هذا إلى سهولة تركيز أشعة الليزر كحزمة ضوئية في نقطة أو تشتيتها إلى مساحات كبيرة باستخدام العدسات البصرية ولا تتأثر أشعة الليزر بالمجال المغناطيسي للعينة المراد لحامها وكما يمكن لحام المواد التي يصعب لحامها بالطرق الاعتيادية مثل التيتانيوم والكوارتز[4,5] هنالك عدة طرق للحام المعادنِ والسبائكِ تَستعمل حالياً في طب الأسنان ومن هذه السبائك(نيكل – كروم- تيتانيوم) ، (كوبلت – كروم) [6]،(فضة – بلاديوم) [7]، تعد سبائك معدنِ كروم كوبالتِ من السبائك الغير ثمينة حيث تم استخدامها في الثلاثينيات في طب الأسنان[8]تستعمل بكثرة لصب الأجهزة السنية مثل قواعد الطقوم، تراكيب الطقم الجزئي وأحيانا في صنع أنواع معينة من أعمال الجسور الثابتة، كما وتستخدم كفرز سني وفي الجراحة التقويمية شكل صفائح او لوالب بسبب ملكياتِهم الميكانيكيةِ القويةِ، مقاومة تآكلِ عاليةِ وكلفةِ منخفضةِ [9,10]

المواد وطرق العمل

أ- **الجانب العملي**

أولا : استخدمت الطريقة التقليدية بطريقه لحام السبيكة المكونة من كوبلت – كروم وضعت المواد مخلوطة في بوتقة معدة لذلك داخل خلية مفرغة لتكون معزولة عن الهواء, سمك السبيكة (3mm) وقد تم اللحام على سطح السبيكة، وسوف نرمز لها بالجداول برمز A.

ثانيا :أجريت عملية لحام سبيكة (كوبلت _ كروم) بالفراغ تحت ضغط جوي 10^{-2} (mbar) بطاقات ليزر مختلفة وتم دراسة تغير العمق مع الطاقة وان منظومة اللحام تتألف من حاوية من البراص لوضع العينة المراد لحامها بداخلها وغطاء علوي من الزجاج لغرض نفوذ أشعة الليزر إلى العينة وتحوي كذلك على مضخة تفريغ . واستخدم ليزر نبضي من نوع -Nd

YAGبزمن نبضة ثابت قدر(8 msec) وطول موجي µm (1.06)، فان طاقة أشعة الليزر المنبعثة من هذه المنظومة بالإمكان تعييرها بواسطة تغير الطاقة الداخلة ، وبالجداول نرمز لها B .

ب- تحضير عينات السبيكة للفحوصات

بعد تحضير عينات قياسيه وذالك لإجراء اختبارات عليها بعد عمليه اللحام ، حيث تؤخذ العينة الملحومة،ويقطع جزء صغير منها يشمل منطقة اللحام وجزئي منطقة الأساس وتم وضعها في قالب من راتنج الايبوكسي (Epoxy resin)، ثم جرى عليها عملية التنعيم مع التبليل بالماء لمنع ارتفاع درجة حرارة العينات ، وبعد ذالك غسلت العينة بالماء المقطر ثم الكحول المثلي وأخيرا جففت لتصبح جاهزة للفحص .

ج- قياس عمق وقطر اللحام

بعد إجراء عملية اللحام حيث يتم قياس عمق اللحام، وكذالك يتم قياس قطر التفاعل باستعمال المايكروميتر المربوط مع المجهر الضوئي.

النتائج والمناقشة

أ- نتائج اختبار الشد:

تمت عملية اختبار الشد للعينة التي أبعادها(0.5mmx3mmx 20mm)الملحومة والأساس بواسطة جهاز اختبار الشد ويمكن تعريف هذا القياس بأنه قوة الشد المسلطة لسحب العينة طوليا وإحداث القطع ، وتجري هذه الطريقة بتثبيت العينة بمقطع عرضي منتظم من طرفيها ويتم تسليط قوة للسحب بالتدريج وبالاتجاه الطولي لحين حدوث القطع ، عندها يتم حساب مقاومة الشد ويؤخذ بنظر الاعتبار مساحة المقطع العرضي للعينة.

يوضح الجدول(1) نتائج قيم إجهاد الشد للعينات ،يعد اختبار الشد من الاختبارات الإتلافية (Destructive testing) التي تتعرض لها العينات ، فيتبين أن قيمة الشد لسبيكة B أعلى من السبيكة A، حيث إن اللحام بطريقة الليزر بسبب التبريد السريع وكذالك عند إجراء الشد على العينة الملحومة كان الكسر في منطقة الأساس وهذا يعطي دلاله على أن قوة تماسك جزئيات منطقة اللحام أقوى من جزئيات منطقة الأساس ،باختيار زمن النبضة الأمثل msec(8) ,لغرض الحصول على عملية صهر دون حدوث تبخر ولحام بكفاءة عالية ، لذالك امتلكت منطقة اللحام مقاومة شد عاليه. أما اللحام بطريقة التقليدية، فإنها تأخذ فتره طويلة

لكي تتجمد فيكون التبريد غير تلقائي يؤدي إلى تشقق سطح نقطة اللحام وإلى ضعفها. من ثم حدوث تركيب مسامي والذي يضعف من اللحام فبعد إجراء اختبار الشد على العينات كان الكسر في منطقة اللحام ،مما يدل على قوة تماسك جزيئات منطقة الأساس أقوى من تماسك جزيئات منطقة اللحام,

جدول (1): نتائج مقاومة الشد

السبائك	مقاومة الشد (N/mm^2)
السبيكة **A**	132.4
السبيكة **B**	151.08

ب- نتائج الصلادة

لقد جرى فحص الصلادة السطحية باستخدام ماسة فيكرز لجهاز(Vickers Hardness) وتبدأ عملية القياس من مركز منطقه اللحام, تتضمنها المنطقة المتأثرة حراريا . فقط كان الوزن المستخدم للعينة (60gm) وزمن التسليط (10s) . واجري الاختبار للأركان الأربعة للعينة عند مسافة (μ m 50) من كل حافة وتم حساب معدل القيمة وعدد فيكرز للصلادة باستخدام العلاقة التالية :

$$HVN= (2F \, Sin \, \varphi)/ D^2 \quad --- (1)$$

حيث تمثل : HVN : عدد فيكرز للصلادة

F: الحمل المسلط (Kg/mm2)

Φ : الزاوية الراسية بين الوجهين المتقابلين للقاعدة الرباعية للهرم وتساوي (136/2)

D: المعدل الحسابي لقطري المضلع الرباعي (mm) ,[5]

يوضح الجدول (2) قيم الصلادة عند مركز منطقة اللحام وعرض اللحام وعرض المنطقة المتأثرة حراريا ظهرت النتائج السبيكة B أعلى من صلادة A،يعزى السبب في ذلك إلى معدل التبريد وسرعة اللحام ، وأيضا إن تبريد السريع الذي أدى إلى تكوين حبيبات طولية وان انزلاق الحبيبات مقيدا وهذا يزيد من الصلادة.والى التوزيع المتجانس لطاقة شعاع الليزر عما هو بالطريقة التقليدية.

جدول (2): نتائج الصلادة وخصائص اللحام لسبيكة (A) . و (B)

السبيكة	عرض اللحام (mm)	عرض HAZ (mm)	صلادة مركز منطقة اللحام H.V
سبيكة معاملة بالطريقة الاعتيادية(A)	201.91	0.86	0.28
سبيكة معاملة بلليزر(B)	182.4	0.63	0.34

ج- نتائج عمق اللحام وقطر التفاعل :

نلاحظ من الجداول (3),(4) نتائج عمق اللحام لعدة قيم طاقية للسبيكة A,B ، فان زيادة الطاقة تؤدي إلى زيادة عمق اللحام من خلال النتائج عمق اللحام يعتمد على عدة عوامل أهمها ، الانتشارية الحرارية وانعكاسية السطح[11] .أما قطر التفاعل توضحها الجدول (5) و (6),فعند سقوط حزمة الليزر على سطح المعدن ما تحدث عملية الانصهار ومن ثم التصلب في هيئة بقعة لحام متصلبة وذالك بسبب الخصائص الحرارية للمادة ومن الجداول نلاحظ أن زيادة الطاقة تؤدي إلى زيادة قطر التفاعل والسبب في ذالك هو زيادة انفراجية الحزمة حيث إن توزيع المجال الكهرومغناطيسي عند الطاقات القليلة يكون بالنمط المستعرض الكاوسي حيث يمتاز بأقل انفراجية.[3]

جدول (3):نتائج عمق اللحام للسبيكة A

العمق (mm)	الطاقة(Joul)
0.31	9.31
0.346	10.21
0.41	11.75
0.47	13.25
0.55	15.02

جدول (4): نتائج عمق اللحام للسبيكةB

العمق (mm)	الطاقة (Joul)
0.51	10.54
0.54	11.47
0.69	15.74
0.75	17.32
0.79	18.42

د–التركيب المجهري :

تم استخدام المجهر من نوع(OLYMPUS) لتصوير سطح العينات المختلفة بقوة تكبير(X100),لغرض دراسة التركيب المجهري للسطح.

توضح الأشكال (1)،(2) الصور الناتجة من هذا الفحص فاظهر الفحص المجهري للسبيكة (A) بان سبيكة اللحام لا ترتبط بشدة مع الأجزاء الملحومة. عكس السبيكة (B).أن المنطقة المتأثرة بالليزر تعتمد بصورة رئيسيه على التبؤر حزمة الليزر والطاقة الكلية الممتصة والتوصيلية الحرارية للمعدن[3].لان انتقال الطاقة يعتمد بصورة كبيرة على خلو سطح المعدن من طبقات الاوكسيد والمواد غير العضوية [11] .

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داء التدخين وتأثيره على فعالية إنزيمـــات ALP,GPT,GST ومـــا يرافقه في إحداث أمراض الكبد المختلفة

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ABSTRACT

This research is designed to study the impact of smoking on the effectiveness of enzyme GST (Klotathaion - Ace - Transeveriz) and the effectiveness of an enzyme, GPT (Klotamet Baiarovi Transeveriz) and the effectiveness of enzyme ALP (enzyme phosphate base) Smoking has been divided into three groups. Group A ((A is a group of smokers for a period of time (2-15) in number (30) people,Group B ((B is a group of smokers for a period of time more than (15) years and over number (30) people. The third group (C) representing a control number (30) people from non-smokers.

We have found an increase in the level of ALP, GPT, GST in direct proportion to the length of time with smokers and is therefore considered an indicator of damage in some of the body especially the liver through the high effectiveness of the enzyme GPT. The effectiveness of the high GST enzyme is considered a sign of increased toxicity in red blood cells and liver cells.> vertauschen

الملخص

تمت دراسة تأثير التدخين على فعاليـــة إنزيم GST (كلوتاثايون-اس-ترانسفريز) وفعالية إنزيم GPT (كلوتاميت بايروفيت ترانسفريز) وفعالية إنزيم ALP (الإنـــزيم الفوسفاتي القاعدي) تم تقـــسيم المـــدخنين على ثلاثة مجاميع .

المجموعة الأولى (A) تمثـــل مجموعـــة المدخنين لفترة زمنيه (2-15) سنة العدد (30) شخص

المجموعة الثانيـــة (B) تمثـــل مجموعـــة المدخنين لفترة زمنيه أكثر من (15) سنة فما فوق العدد (30) شخص .

المجموعة الثالثة (C) تمثـــل مجموعـــة السيطرة العدد (30) شـــخص مـــن غيـــر المدخنين .

لقد وجد ارتفـــاع فـــي مـــستوى فعاليـــة إنزيمـــات ALP,GPT,GS T يتناســـب طرديا مع طول الفترة الزمنية المـــدخنين وذلك يعتبر مؤشرا لحدوث أضـــرار فـــي بعض أعضاء الجسم وخاصة الكبد مـــن خلال ارتفاع فعالية أنـــزيم GPT . أمـــا ارتفاع فعالية إنزيم GST فيعتبـــر دلالـــة على زيادة السمية في كريات الدم الحمراء والخلايا الكبدية .

المقدمة

يعـــد داء التـــدخين مـــن المـــشاكل الاقتصادية والصحية للإنسان، و خاصـــة أمراض سرطان الرئة [1]و أمراض القلب وأمراض الكبد وتشير منظمـــة الـــصحة العالمية بموت شخص كـــل ســـت ثـــوان ونصف بسبب التدخين،[2]

فقد وجد أن التدخين يتسبب فـــي زيـــادة السمية لكثير من الأدوية بـــسبب تحفيـــزه لبعض الإنزيمات التي تتعامل مـــع هـــذه الأدوية بالكبد و يتسبب أيضا التدخين فـــي أضعاف قدرة الكبد على التخلص من المادة الضارة التي تدخل الجسم ومن الممكن أن تؤثر أيضا في مستوى الأدوية التي يتناولها المريض لعلاج مرض الكبد. وكذلك وجد ارتباط بين التدخين و سرطان الكبد.

ويزيد التدخين أيضا من شدة التهاب الكبد الناتج عن الفيروسات كما فـــي مرضـــى الالتهاب الفيروسي المزمن نتيجة لفيروس "سى" و ينعكس ذلك على مستوى إنزيمات الكبد فنجدها أعلى لدى المرضى المدخنين مقارنة بغيرهم من غير المـــدخنين. وقـــد وجد أن درجة الالتهاب و مرحلة التليـــف الناتج عن الفيروس الكبد ى "سى" أشد في المدخنين عن غيرهم . ومن المعروف أن المدخنين لا يعانون من نقص الهيموجلوبين و ذلك لاستمرار إنتاجه في النخاع بـــسبب إحساس الأنسجة بنقص في الأوكـــسجين, المعادن الثقيلة تُسبّبُ ضرر بـــالغ بـــشكل ملحوظ على الصحةِ الإنسانيةِ في الحقيقة، وضّحتْ بَعْض الاستطلاعات بأنّ محتويات بَعْض المعـــادنِ الثقيلـــةِ الـــسامّةِ، [2,3] ويدخل الحديد في تركيب الهيموجلوبين لذا فان مستوى الحديد بالدم يكون مرتفعا لدى المدخنين. ومن المعروف أيضا أن الحديد سام للكبـــد إذا ارتفعـــت مـــستوياته فـــي الأنـــسجة. ومرضـــى الفيـــروس "ســـى"

المدخنين يستجيبون أقل للعلاج بمضادات الفيروس.

لذا على مريض الكبد ان يتوقف عن التدخين ويعتبر من الخطوات العلاجية الهامة التي لا تكلف وربما تقلل من الحاجة إلى بعض الأدوية الأخرى وأيضا فان التوقف عن التدخين يزيد من نسب نجاح العلاج بمضادات الفيروس مثل الانترفيرون

يحتوي دخان السجائر خليطا Mixture كيميائيا معقدا للغاية وخطيرا على صحة الإنسان وعلى كافة عناصر البيئة ، فهو يحتوي على أكثر من 3800 مادة كيميائية سامة ، ومن أهمها نذكر أول أكسيد الكربون CO وكبريتيد الهيدروجين H_2S والأمونيا NH_3 والفورمالدهايد HCHO والأسيتالدهايد CH_3CHO وسيانيد الهيدروجين HCN [4]، بالإضافة إلى طائفة كبيرة من الأحماض المختلفة ، من أهمها حامض الكربونيك H_2CO_3 وحامض النيتريك HNO_3 وحامض الخليك CH_3COOH وحامض الفورميك HCOOH [5].

طريقة العمل

1- قياس فعالية إنزيم GST في كريات الدم الحمراء[6,7]

تعتمد هذه الطريقة على تفاعل الكلوتاثايون مع 1- كلورو- 2- 4- داي نايترو بنزين ليكون 2- 4- داي نايترو فنيل كلوتاثايون ويقاس على طول موجي (340 nm)

2- قياس فعاليه إنزيم GPT في مصل الدم [8]

تعتمد هذه الطريقة على تفاعل بين L-alanin مع α-oxoglutarate لينتج L-glutamate + بايروفيت ويتفاعل البايروفيت مع 2 - 4- داي نايترو فنيل – هايدرازين يكون معقد لوني يقرأ على طول موجي (546nm) .

3- قياس فعالية إنزيمALP في مصل الدم[9,10] .

تعتمد هذه الطريقة على تحلل فنيل فوسفيت إلى فينول + فوسفيت ويتفاعل الفينول مع امينو-4-انتي بايرين بوجود Potassium ferro cyanite وقرأ عند طول موجي (510nm) .

4- تقدير تركيز الهيموكلوبين [11]طريقة سيانو ميثوهيموكلوبين

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النتائج والمناقشة

نلاحظ في الجدول رقم (1) ارتفاع في مستوى الفعالية النوعية لإنزيم GST مع طول الفترة الزمنية للمدخنين . إنزيم GST يلعب دورا أساسيا في تخفيف الخصائص السامة للمركبات الغريبة حيث يرتبط GSH مع الأجسام الغريبة بواسطة هذا الإنزيم وعندما تزداد الأجسام الغريبة (Xanthobiotics) والتي تشمل الملوثات الصناعية , الجذور الحرة . تزداد فعالية إنزيم GST للتخلص من تلك الأجسام الغريبة ويوجد هذا الإنزيم بشكل كبير في الكبد[12.13]

جدول رقم (1) يحدد معدل الانحراف القياسي

والمعدل ومستوى فعاليه إنزيم GSTعند مجموعة السيطرة ومجاميع المدخنين

المجاميع	GST(U\gHb			
	العدد	±S.D	Mean	T
C	30	0.05	0.79	_
A	30	0.08	0.91	P<0.05
B	30	0.99	1.25	P<0.001

(A) تمثل مجموعة المدخنين لفترة زمنيه (2-15) سنة

(B) تمثل مجموعة المدخنين لفترة زمنيه اكثر من (15) سنة فما فوق.

(C) تمثل مجموعة السيطرة من غير المدخنين

نلاحظ في جدول رقم (2) ارتفاع في مستوى فعالية الإنزيم GPT مع طول الفترة الزمنية للمدخنين . تزداد فعالية إنزيم GPT كلوتاميك بايروفيت الناقل والذي يوجد بنسبة كبيرة في أنسجة الكبد لهذا يعتبر كدليل مهم في الكشف عن أي ضرر يصيب الكبد [14]

جدول رقم (2) يحدد معدل الانحراف القياسي

والمعدل ومستوى فعاليه إنزيم GpTعند مجموعة السيطرة ومجاميع المدخنين

GpT(U\L				المجاميع
T	Mean	±S.D	العدد	
-	10.1	1.3	30	C
P<0.05	17.8	3.2	30	A
P<0.001	37.2	5.7	30	B

A,B.Cكما في جدول 1

نلاحظ من جدول رقم (3) ارتفـــاع فـــي مستوى فعالية إنزيم ALP مع طول الفترة الزمنية للمدخنين حيث يعتبر إنزيم ALP كدليل مهم في الكشف عن الأضرار التـــي تتعرض لها أنسجة العظام ولاســـيما فـــي احتمال ترسب في نخاع العظم [15]

جدول رقم (3) يحدد معدل الانحراف القياسي

والمعدل ومستوى فعاليه إنزيم ALPعند مجموعة السيطرة ومجاميع المدخنين

ALP (kingU\DL)				المجاميع
T	Mean	±S.D	العدد	
-	10.2	1.25	30	C
P<0.05	19.4	3.9	30	A
P<0.001	35.6	6.3	30	B

A,B.Cكما في جدول 1

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Sambrook J, Russell DW. 2001. Molecular Cloning: A Laboratory Manual, 3rd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

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