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

Volume 14 / Number 4 / October-December 2020

Indian Journal of Forensic Medicine & Toxicology

Website: www.ijfmt.com

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

Indian Journal of Forensic Medicine & Toxicology

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

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Editor

Dr. R.K. Sharma
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Printed, published and owned by

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

Published at

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

Correlation Ship between Some Hematological Parameters and Smoking for Students

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Abstract

This study including collected 172 blood sample from AL-Kafeel University college, these samples including 102 sample to male and 70 sample for female in which students samples were 53 for smoker and 119 non-smoker , to evaluate some of parameters such as ABO , RH , blood pressure and clotting time .

The results showed blood group A^+ was more frequent which represent 33.7 % followed by O^+ blood group which represent 25.6% while B^- was the last ,in other results displayed AB^- and RH $^+$ was more frequent than RH $^-$. Systolic and diastolic pressure examined was demonstrated there were mild hypertension in blood pressure between samples of studied, while result gloating time was exhibited range 30 sec to 3 minute .

Keywords: *blood group, duration of smoking, Blood pressure and Clotting time*

Introduction

Blood pressure is a cardiovascular measurement with dynamic characteristics that can be influenced by a number of internal and external factors. Hypertension is classified as either primary or secondary. It is primary when no medical cause can be found to explain the raised blood pressure. This type represents between 90 and 95% of hypertension cases(1). Secondary hypertension represents approximately 10% of all hypertension cases. Identifiable underlying causes of secondary hypertension are kidney disease, renal hyperaldosteronism, and pheochromocytoma. Secondary hypertension has specific therapy; it is potentially curable and often distinguishable from primary one on clinical grounds(2). The term high blood pressure mean each blood pressure above 120/80 mm Hg. High blood pressure was known as the “silent killer” due to the large destruction caused to the blood vessels (3) ,whereas hypertension refers only to pressures of 140/90 mmHg and above(4).

A systolic blood pressure of fewer than (90) millimeters of mercury (mm Hg) or diastolic of less than 60 mm Hg was usually considered to be hypotension(5).

The blood pressure involves two numbers. The top number indicates the force of shrinkage of the heart’s main section, the left ventricle, and the lower number agrees with the resistance to blood flow in the arteries(6) .There are convinced risk factors that we have no control over for instance: genetics, age, sex and race according to Casey and Benson, (2006).

The antigens of the ABO blood group system (A, B and H determinants) are complex carbohydrate molecules expressed on red blood cells and on a variety of other cell lines and tissues (7).

Smoking is an important cardiovascular disease risk factor, but the mechanisms linking smoking to blood pressure are poorly understood(8) .

Materials and Method

Sample collection

This study was involve collection of 172 blood

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sample of Al-Kafeel university students, then some of tests was accomplished.

Blood pressure determination:

Check that the patient's arm is not restricted by any tight clothing. Support the arm with a pillow, ensuring that it is level with the person's heart (midst Ernst level). than ,Wrap the BP cuff around the patient's bare arm. The cuff should be positioned 2-3cm above the brachial artery , Ask the patient not to talk during the procedure. Locate the radial pulse. Inflate the BP cuff by pumping the cuff bulb, until the radial pulse can no longer be felt. Note the reading on the dial. This figure is the estimated systolic pressure, Deflate the BP cuff completely and wait for 15-30 seconds. Inflate the cuff again to 20-30mmHg above the predicted systolic BP.

Clotting time Procedure:

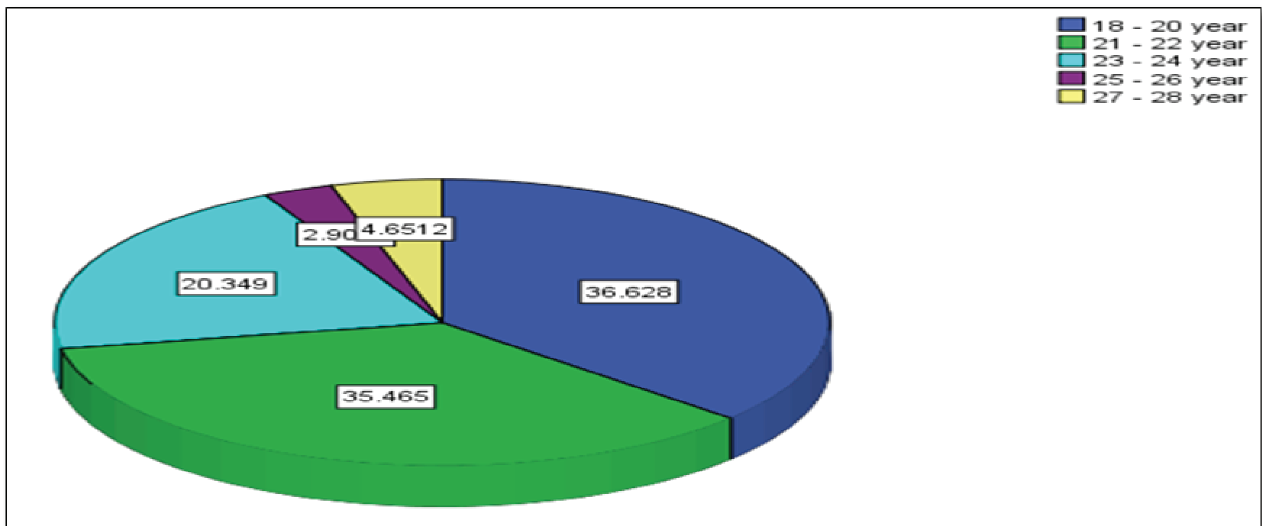
Slim the finger with spirit than allow the spirit to dry, Pierced the finger by lancet ,remove the first drop of blood, Squeeze the finger to obtain a larger drop of blood and fill the capillary tube with blood, The capillary tubes are sealed plastic and immersed in water bath at 37 centigrade After one minute start breaking small pieces of the capillary tube every30 second until a fibrin thread is seen between the two broken ends, By these methods the normal clotting time is 5 to 10 minutes at 37co

Blood group Procedure :

A clean and dry glass slide was divided into two sections by using a glass marking pencil. The sections were labeled as anti-A and anti-B to recognize the antisera, Place one drop from anti-A serum and one drop from anti-B serum in the center of the corresponding section of the slide. Mix antiserum with blood by using a separate corner of a slide for each section over an area about 1 inch in diameter. By tipping the slide forwards and backwards, examine for agglutination after exactly two minutes .

Results and Discussion

The figure(1) show the percentage of the sample by age, where the percentage of age group 18-20 years was a highest percentage which represent 36.6% followed by the age group 21-22 years, where it reached 35.5% followed by the age group. Followed by the age group 21-22 years and amounted to 35.5% followed by the age group 23-24 years, where it reached 20.3%, followed by the age group 27-28 years, reaching 4.7%.



The figure(1) shows the percentage of the sample by age .

This study was proved that the blood type that more frequently was A+ , followed by O+. While the least frequently was B- and AB-. And the Rh + was more frequently than Rh- this result agreed with[9].

Table (1) the distribution of Rh

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	A+	58	33.7	33.7	33.7
	B+	38	22.1	22.1	55.8
	AB+	17	9.9	9.9	65.7
	O+	44	25.6	25.6	91.3
	A-	5	2.9	2.9	94.2
	B-	2	1.2	1.2	95.3
	AB-	2	1.2	1.2	96.5
	O-	6	3.5	3.5	100.0
	Total	172	100.0	100.0	

Table and figure (2) shows the relationship of blood clotting with age coagulation period 30 sec. was the highest percentage in the age group 18-20 years, where it reached 6.3%. The frequency of 1min was in the age group 25-26, where it reached 20%. And the period of 1.5m, which reached the highest percentage in the age group 21-22 years. And the period of 2m was the highest proportion in the age group 18-20 years. And the period of 2.5m was the highest percentage in the age group 27-28 years. The period of 3m was the highest percentage in the age group 27-28. In other study Blood coagulation capacity rises with age in healthy individuals, seemingly because of increases in the plasma concentration of most procoagulant factors. This phenomenon may play an important role in the advancing age–associated increase of cardiovascular diseases and thrombosis [10].

Table (2) shows the relationship of blood clotting with age

age group			Frequency	Percent	Valid Percent	Cumulative Percent
18 - 20 year	Valid	30 sec	4	6.3	6.3	6.3
		1 min	8	12.7	12.7	19.0
		1.5 min	10	15.9	15.9	34.9
		2 min	30	47.6	47.6	82.5
		2.5 min	4	6.3	6.3	88.9
		3 min	7	11.1	11.1	100.0
		Total	63	100.0	100.0	
21 - 22 year	Valid	30 sec	3	4.9	4.9	4.9
		1 min	11	18.0	18.0	23.0
		1.5 min	11	18.0	18.0	41.0
		2 min	23	37.7	37.7	78.7
		2.5 min	6	9.8	9.8	88.5
		3 min	7	11.5	11.5	100.0
		Total	61	100.0	100.0	

***Cont..* Table (2) shows the relationship of blood clotting with age**

23 - 24 year	Valid	30 sec	1	2.9	2.9	2.9
		1 min	3	8.6	8.6	11.4
		1.5 min	5	14.3	14.3	25.7
		2 min	13	37.1	37.1	62.9
		2.5 min	8	22.9	22.9	85.7
		3 min	5	14.3	14.3	100.0
		Total	35	100.0	100.0	
25 - 26 year	Valid	1 min	1	20.0	20.0	20.0
		2 min	2	40.0	40.0	60.0
		2.5 min	1	20.0	20.0	80.0
		3 min	1	20.0	20.0	100.0
		Total	5	100.0	100.0	
27 - 28 year	Valid	2 min	3	37.5	37.5	37.5
		2.5 min	3	37.5	37.5	75.0
		3 min	2	25.0	25.0	100.0
		Total	8	100.0	100.0	

Table and figure(3)shows the relationship of coagulation to sex ,where the period of coagulation 30.sce was higher in females, where it reached 5.7%. At the same time, the percentage of females was high at 15.7%. to 1.5min, it was 17.1% In the period of 2 min, the percentage of females was highest, reaching 50.0% At 2.5%, the percentage of male was higher at 16.7% and at 3 min the percentage of male was higher at 18.6.

Table(3) shows the relationship between coagulation and sex

Gender			Frequency	Percent	Valid Percent	Cumulative Percent
Male	Valid	30 sec	4	3.9	3.9	3.9
		1 min	12	11.8	11.8	15.7
		1.5 min	14	13.7	13.7	29.4
		2 min	36	35.3	35.3	64.7
		2.5 min	17	16.7	16.7	81.4
		3 min	19	18.6	18.6	100.0
		Total	102	100.0	100.0	
Female	Valid	30 sec	4	5.7	5.7	5.7
		1 min	11	15.7	15.7	21.4
		1.5 min	12	17.1	17.1	38.6
		2 min	35	50.0	50.0	88.6
		2.5 min	5	7.1	7.1	95.7
		3 min	3	4.3	4.3	100.0
		Total	70	100.0	100.0	

Table and figure (4) relationship between the coagulation and smoking where the duration of coagulation 30sce, where it reached the highest proportion of non-smokers, where it reached 5.0%. The highest rate was 1min in smokers (17.0%). The ratio of 1.5min was the highest rate for non-smokers, at 16.8%. The ratio of 2min was the highest percentage of non-smokers, reaching 44.5%.The rate of 2.5min was the highest in smokers, reaching 17.0%. and during the period of 3 min where it reached the highest proportion of smokers, reaching 17.0

The effect of smoking strength on the degree of damage of coagulation cascade motionless remains uncertain, while effects of cigarette smoking on the normal hemostasis thru influencing the coagulation pathways(11).

Table (4) relationship between the coagulation and smoking

smoking status			Frequency	Percent	Valid Percent	Cumulative Percent
Smoker	Valid	30 sec	2	3.8	3.8	3.8
		1 min	9	17.0	17.0	20.8
		1.5 min	6	11.3	11.3	32.1
		2 min	18	34.0	34.0	66.0
		2.5 min	9	17.0	17.0	83.0
		3 min	9	17.0	17.0	100.0
		Total	53	100.0	100.0	
non smoker	Valid	30 sec	6	5.0	5.0	5.0
		1 min	14	11.8	11.8	16.8
		1.5 min	20	16.8	16.8	33.6
		2 min	53	44.5	44.5	78.2
		2.5 min	13	10.9	10.9	89.1
		3 min	13	10.9	10.9	100.0
		Total	119	100.0	100.0	

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

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

Funding: Self-funding

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Impact of Digoxin on Endocrine Glands in Male Rats

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Abstract

This study is performed to provide the pathological changes of thyroid gland, pancreas and adrenal gland that occurred due to exposure to the digoxin drug. The study uses twenty four adult male rat randomly divided into 3 equal groups as follows: G1 control group treated with distilled water, G2 dosed with 0.3mg/kg digoxin and G3 dosed with 0.9mg/kg digoxin given orally by gavage daily for 60 days. All animals were sacrificed at the end of experiment and organs were collected in 10% buffered formalin for histopathological evaluation. Result reveals dilation of thyroid follicles with flattened follicular epithelium which increase with high dose group digoxin in comparison with control. Adrenal gland showed moderate atrophy of cortical layers especially zona reticularis with hyperplasia of medulla cells probably of digoxin like ouabain-producing cells, on the other hands pancreas doesn't reveal remarkable changes except congestion of vessels in high dose group compared to control.

Keywords: *male rats; endocrine glands; pathological study and digoxin*

Introduction

Digoxin is one of the old medicine for heart failure and still the drug of choice in this condition[1,2], several drug have been developed but for treatment of symptoms of disease to complete cure and these drug is too expensive without any benefit that can be added above digoxin to replace it [3]. Digoxin increase force of contraction of myocardial muscle cells that is inotropy which exerted through blockage of Na^+-K^+ ATPase pump[4]. The most problems that accompanied digoxin it is narrow therapeutic window which make it toxicity sever to be occur so any mistake of drug toxicity denote [5,6]. Ouabain is an endogenous substance similar to digoxin that is produced from adrenal medulla cells. Ouabain previously used for treatment of heart failure[7]. Because digoxin is prescribed for all aged group patients so this study is designed to investigate the histopathological changes of digoxin on thyroid gland, pancreas and adrenal gland.

Materials and Methods

Experimental animals

This study administered 24 adult male rat divided randomly into 3 groups as follows; G1 is control group processed with distill water, G2 treated with 0.3mg/kg digoxin and G3 treated with 0.9mg/kg digoxin orally by gavage daily for 60 days. The animals were kept for adaptation for 3 weeks before experiment at the laboratory animal house of College of Pharmacy – University of Basrah. Animals were housed as eight rats in each cage under conditions (Mechanical ventilation, 12\12 hours light on, pellet, tap water and room temperature 24 ± 2ºC).

Histopathological study:

After 60 days of digoxin dosing, all rats were sacrificed and organs collected in 10% formalin for histopathological processing [8] and stained with hematoxylin and eosin according to Drury [9], to be assessed by light microscope.

Results

Histopathology of control group

Thyroid gland shows normal thyroid follicles apparently of different sizes with cuboidal compact

epithelium and homogenous pinkish colloid material. Pancreas show normal pancreatic glands and in between shows islets of Langerhans in the vicinity of blood vessels. Adrenal gland shows two layer outer one represent adrenal cortex showing three layers including zona glomerulosa, zona fasciculata and zona reticularis, the other layer resembling adrenal medulla showing medullary cells which appear similar to neuronal bodies. Showed in figure1.

Histopathology of low dose group

Thyroid gland showed dilated thyroid follicles with flattened follicular cells filled with thick colloid material, early stage of hypothyroidism and some areas of the glands appear unchanged. Pancreas appear with un remarkable changes showing exocrine glands and in between islets of Langerhans. Adrenal cortex appear atrophied with blurred layers and hyperplasia of the adrenal medulla which occupies most of adrenal tissue. Showed in figure 2.

Histopathology of high dose group

Thyroid gland with moderate to severe flattening of follicular epithelial cells with engorgement of the follicular lumen by faint colloid material. Pancreatic islets of Langerhans show unremarkable pathological changes compared to control slides and there is only congestion of blood vessels. Adrenal gland shows moderate atrophy of adrenal cortical layers especially the zona reticularis with hyperplasia of adrenal medullary cells which appears hypercellular studied with neuron like cells. Showed in figure 3.

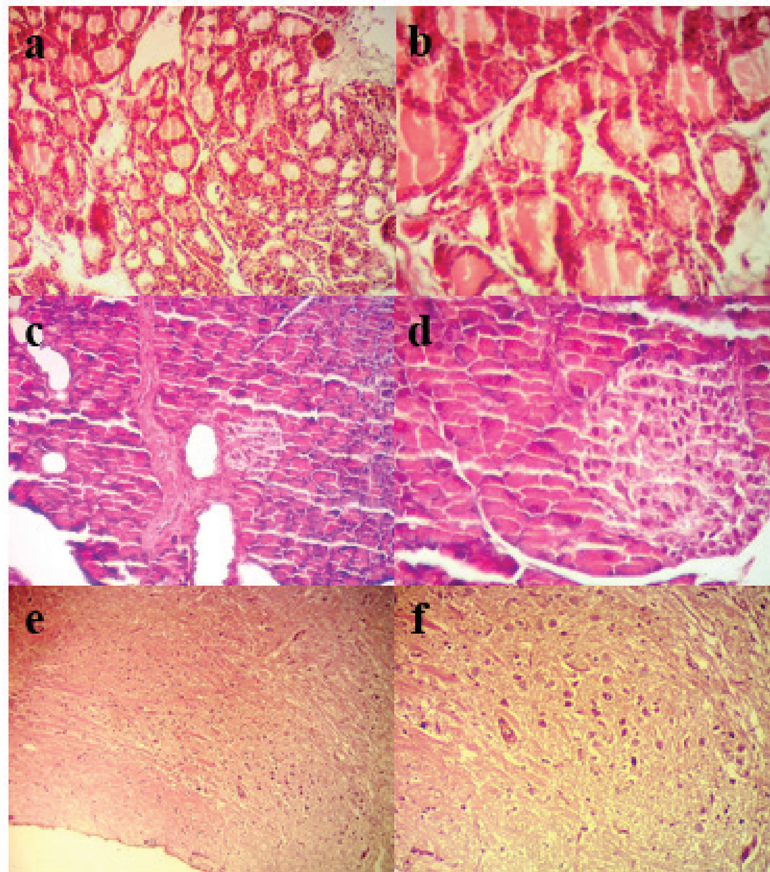

Figure 1: Control group, show normal architecture of thyroid follicles (a 200X &b 400X), pancreas show exocrine gland & islets of langerhance (c 200X &d 400X) and adrenal gland show cortex layers with medulla (e 200X &f 400X).

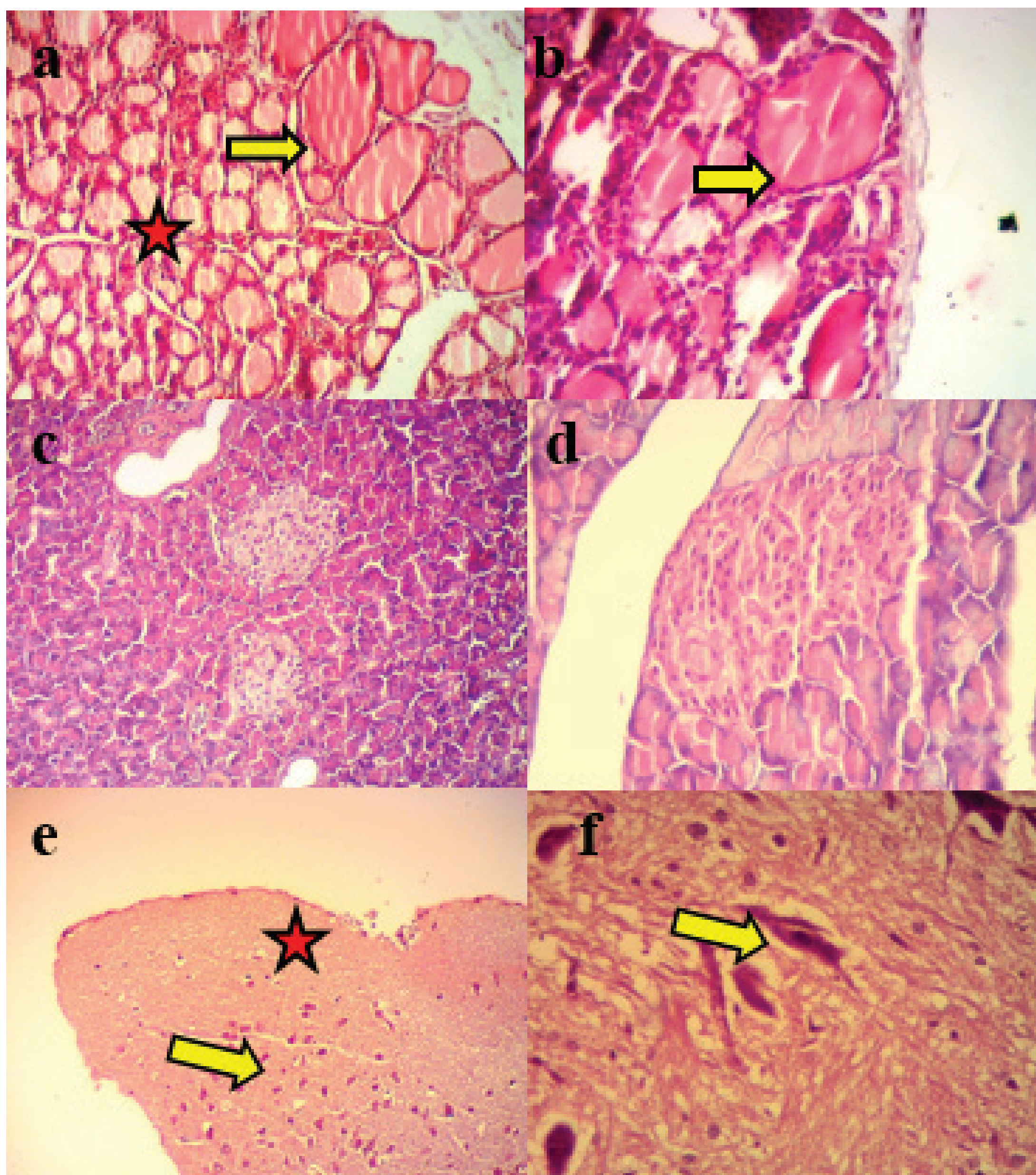

Figure 2: Low digoxin group, show dilation of thyroid follicles (yellow arrow) and some area without changes (red star) (a 200X &b 400X), pancreas don't show any changes in exocrine gland & islets of langerhance (c 200X &d 400X) and adrenal gland show atrophy of cortex layers (red star) with hyperplasia of medullary cells (yellow arrow) (e 200X &f 400X).

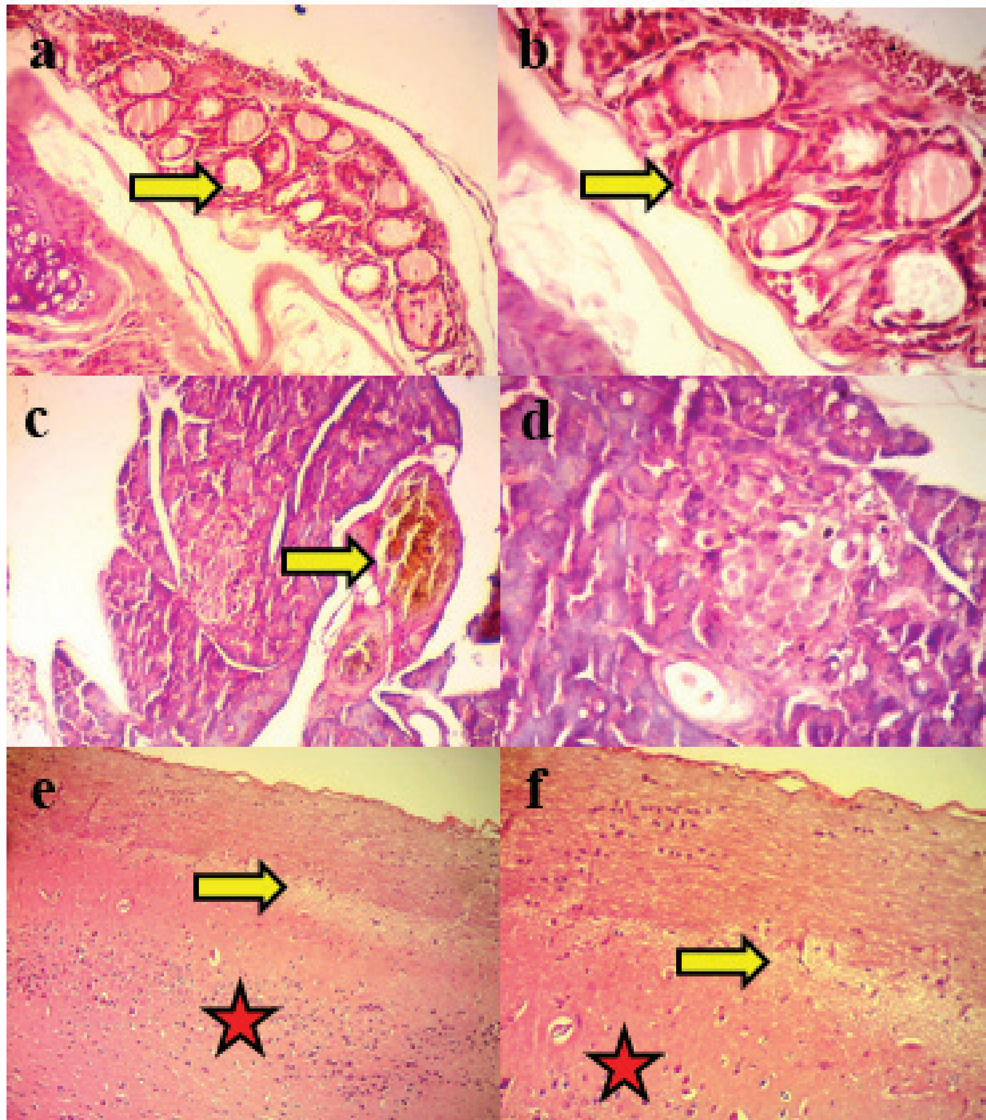

Figure 3: High digoxin group, show moderate to sever flattening of thyroid follicle epithelium filled with colloid (yellow arrow) (a 200X &b 400X), pancreas show unremarkable changes except congestion of blood vessels (yellow arrow) (c 200X &d 400X) and adrenal gland show atrophy of cortex layers especially zona reticularis (yellow arrow) with medullary cells hyperplasia (red star) (e 200X & f 400X).

Discussion

The present study is designed to provide a data base about histopathological changes caused by digoxin drug on thyroid, pancreas and adrenal glands in rats. The control group showed normal architecture of these gland as shown in figure-1. It has been noted that increase dose of digoxin cause more changes in these gland compared to lower dose group, thyroid gland presented as enlarged follicles associated with flattened epithelium which may be related to effect of digoxin on thyroid gland to suppress thyroxin hormone release because of it is anticipated action on myocardium. Digoxin provide positive inotropic effect on the heart, which leads to increase stroke volume, vascular perfusion and improve blood pressure. This might cause a negative feedback on the function of thyroid gland which shows features of hypo functioning thyroid follicles. The present study doesn't reveal significant changes in pancreatic tissue except in high dose digoxin pancreas which revealed congested blood vessels, as shown in figure-3.

Ouabain is an endogenous digoxin-like substance that is produced by adrenal medulla cells and it has a similar mechanism of action to digoxin [7,10]. During heart failure, because digoxin blocks the action of adrenaline effect on the heart, this probably may lead to atrophy of adrenalin producing cells in adrenal medulla which is accompanied by hyperplasia of ouabain synthetic cells. This finding is evident by the histological appearance of hyperplasia of ouabain-producing medullary cells of adrenal gland after digoxin administration which becomes more prominent with increased dosage of digoxin. This result is in line with Takahashi [10], who reported that a monoclonal ouabain antibody was highly distributed in adrenal medulla. Medullary hyperplasia is associated with atrophy of cortical layers of adrenal gland as shown in figures-2 and 3.

Upon reviewing literature regarding the histopathological changes of cardiac glycoside (digoxin), the authors didn't come across any research studying these changes. This research may be the first of it is kind in this field.

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

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

Funding: Self-funding

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Study Some Biochemical Parameters in Beta Thalassemia Major Patients in Males and Females Adults in Baghdad

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Summary

Our study aims to investigate in levels of Ferrtin and IL-23 in beta-thalassemia major patients (males and females), and compared with the control group (healthy individuals). The total number of samples were (88) blood samples, beta-thalassemia major patients (44) and healthy individuals (44). All the samples submitted to ELIZA test to determination Ferrtin and IL-23 in blood samples (males and females) (patients and healthy group). According to our results, showed there are significant difference in and males with beta-thalassemia major and females with beta-thalassemia major in Ferrtin concentration as compared with the control group at (P<0.01). In addition, Our study showed no there are significant difference in and males with beta-thalassemia major and females with beta-thalassemia major in IL-23 level as compared with the control group at (P<0.01). Finally, there is a high increase in Ferrtin concentration of blood samples in males and females, while there are little changes (non-significant) in IL-23 concentration of blood samples in males and females.

Keyword: *Ferrtin, IL-23, beta-thalassemia major***.**

Introduction

Thalassemias are genetic disorders, included decreased production of the hemoglobin (alpha or beta chain or both). Hemoglobin function is to carry the oxygen and formed from two proteins. Decreasing of these proteins leads to the inability of RBC to transfer the oxygen; leads to anaemia. Thalassemia is occurred due to deletion or mutation of some gene [(1) (2).]

Thalassemias have two types (Alpha and Beta). Alpha thalassemia is occurring due to the deletion of the alpha-globin gene or disorder of the synthesis of alpha-globin [(3) (4).] In addition, Beta-thalassemia results from point mutations in the beta-globin gene. It is divided into three categories based on the zygosity of the beta-gene mutation (minor, major and intermediate), these types result in different severity [(5) (6).]

The clinical signs of thalassemia appear before a child's second birthday. Very acute anaemia, furthermore, jaundice, frequent infections, a poor appetite, fussiness, failure to thrive, paleness, and the skin becomes yellow, organs enlargement, acute anaemia required blood transfusions [(7).]

Ferritin is protein work inside the cells. Bacteria, animals, archaea and human can produce Ferritin. Ferritin acts as a buffer control level of iron [3]. Ferritin is present in the cell and secreted at small amounts into the serum. Ferritin is indirect indicator of the total amount of stored iron therefore; the ferritin test is used as a laboratory test for iron concentration [(8) (9).]

Interleukin-23 is a pro-inflammatory mediator formed from two subunits (p19 and p40). It have immunological role in the body. IL-23 is stimuli Th17 cells that have great role in inflammation reaction [(10).]

Our study aims to investigate the relationship and changes in the levels of ferritin and interleukin 23 in the blood of patients with major beta-thalassemia

Material and Methods

Study design:

This prospective study for beta-thalassemia major patients had been conducted from December 2018 until June 2019 in AL Karama teaching hospital in Iraq Baghdad. All the patients had been chosen according to the following inclusion and exclusion criteria included All the patients are suffering from β-thalassemia major, Age more than 18 years old, Non-splenectomized, No obvious clinical infection, hepatitis and unmarried.

Sample size:

The study included eighty-eight (88) patients divided into two groups:

1- The first group (patient): Were forty-four (44) of the β-thalassemia major patients.

2- The second group (control group): was forty-four (44) apparently healthy.

Data collection:

The questionnaire is prepared for all the patients are included data related to name, gender, age, diagnosis of disease and other information.

Venous blood withdrawal:

The venous blood (5) ml was taken from control and patients group included in the study. The collected blood was divided almost (3ml) put in K3-EDTA tubes while other (2ml) keep in plain tubes, then centrifuged for five min at (300) rpm for separation the serum then stored at (-20) C.

Determination of Ferrtin hormone:

Assay Protocol:

1- Adding Pipet Ferritin Standard, control, and samples 25 µl by Pipet into the wells

3- Adding of the biotin Reagent (100) µl on all the wells and mixed.

4- Incubation of the plate for thirteen minute at (25°C)

5- Lifting of the liquid from all wells then washing wells by wash buffer.

6- Adding of the Enzyme Reagent (100) µl on all the wells and mix

7- Incubate for half hour at (25°C).

8- Discarded liquid from all wells and washing wells by using wash buffer.

9- Adding TMB substrate (100) µl on all the wells and incubation fifteen minutes at (25) C.

10- Adding of the stop solution (50) µl on all the wells and mix

11- The absorbance is read at (450) nanometer for fifteen minutes after mixing the stop solution.

Determination of IL-23 hormone:

Assay Procedure:

1- Put control, sample and standard in the wells

2- Adding Sample/Standard dilution buffer (0.1) ml into the zero well

3- Adding diluted sample (0.1) ml into test sample wells

4- Covering and incubation at 37°C for 1.5 hours

5- Left the cover and remove the plate content.

6- Adding Biotin-AB working solution (0.1) ml on the wells, adding of the solution on all wells

7- Covering and incubation at 37°C for one hour

8- Left the cover, and washing it by using wash buffer.

9- Adding of SABC working solution (0.1) ml into all the wells and incubation at 37°C for thirteen minutes

10- Lifting and washing by using wash buffer.

11- Adding TMB substrate (90) µl on all the wells, covering it and incubation at 37°C for thirteen minutes

12- Adding Stop solution (50) µl on all the wells and mixing, the color will be yellow directly.

13- OD Absorbance is read at (450) nanometer by using micro plate reader after mixing with the stop solution.

Statistical Analysis

The Statistical Analysis System software (2012) was applied for detection of the effect of two factors in our study. LSD test was used for compare between the means. Q test was used for compare between percentage (0.01 and 0.05).

Results

According to our results, showed there are significant difference in and males with beta-thalassemia major (3063.26±396.46) and females with beta-thalassemia major (3663.73±523.42) in Ferrtin concentration as compared with the control group (157.24±48.12) and (20.45±3.65) respectively at (P<0.01) as the table (1).

Table (1): Effect of gender and group in Ferrtin

Group	Mean ± SE	
	Male	**Female**
Patients	3063.26 ± 396.46	3663.73 ± 523.42
Control	157.24 ± 48.12	20.45 ± 3.65
T-test (P-value)	1029.10 ** (0.0001)	1329.50 ** (0.0001)

* S: Significant, ** H.S., Highly Significant, NS: Non- Significant.

Based on our study, showed no there are significant difference in and males with beta-thalassemia major (19.58±2.79) and females with beta-thalassemia major (13.99±3.26) in IL-23 level as compared with control group (17.34 ± 1.22) and (21.10 ± 1.04) respectively at (P<0.01) as the table (2).

Table (2): Effect of gender and group in IL-23

Group	Mean ± SE	
	Male	**Female**
Patients	19.58 ± 2.79	13.99 ± 3.26
Control	17.34 ± 1.22	21.10 ± 1.04
T-test (P-value)	7.496 NS (0.550)	8.457 NS (0.096)

*S: Significant, **H.S., Highly Significant, NS: Non- Significant.

Discussion

Many of the changes are occurring in patients with major beta-thalassemia, which include physiological and biological changes. These changes are included levels of hormones and some intermediate compounds in the activation of some reactions in the body such as ferritin and interleukin-23 [11].

Ferritin is a complex protein composed from two subunit heavy chain and light chain. Ferritin is regulate level of iron in the serum [12].

According to our results, showed there are significant difference in and males with beta-thalassemia major and females with beta-thalassemia major in Ferrtin concentration as compared with the control group and respectively at (P<0.01) as the table (1).

Level of the ferritin protein is a reflex level of the iron. In another mean, the ferritin concentration gives a general view of how much iron in the body. If the ferritin level is low, that indicates the iron is low. If the ferritin concentration is high, the iron is high. The high concentration of ferritin is associated with beta major thalassemia due to repeated blood transfusion lead to increasing of iron in the body results in increases of ferritin and that agreement with results of [13] [14].

Interleukin-23 (IL-23) plays an important role in the development and maintenance of T-cell helper, wherever it has a great immune- protective role in the body [15].

Based on our study, showed no there are significant difference in and males with beta-thalassemia major and females with beta-thalassemia major in IL-23 level as compared with the control group and respectively at (P<0.01) as the table (2), There are little increase in males and a little decrease in females.

Some studies such as [16] and [17] showed increasing of IL-23 level, However, another study such as [18] showed little increases non- significant of IL-23 level.

Our study showed no statistical differences in interleukin-23 in males and females as compared with the control group. Perhaps these slight differences between the groups are due to individual factors, un-clinical infection conditions, and other unknown factors.

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

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

Funding: Self-funding

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Biological Activity of Complexes of Some Amino Acid: Review

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Abstract

Schiff bases (SBs) based on amino acid derivative stand for multipurpose ligands that formed by condensing amino acids with carbonyl groups. They are significant in pharmaceutical and medical areas due to their widespread biological actions such as antiseptic, antifungal, along with antitumor actions. Transition metallic complexes resulting from SB ligands with biological activity were extensively experimented in the literature. In this article, we review, in details, about synthesizing and biological performances of SBs along with its complexes.

Keywords: *Schiff bases (SBs), Amino acid, Metal complexes, antimicrobial activity, and antitumor activity.*

Introduction

Compounds having an azomethine cluster (-CH=N-), termed as Schiff bases (SBs) has typically synthesized as a result of condensing primary amine with a carbonyl compound.[1] SBs of aliphatic aldehydes have been somewhat unstable and freely polymerizable. On the other hand, SBs with aromatic aldehydes, including an in effect conjugation system, have been in higher degree of stability [2]. SBs have, in general, particular preparation use, detection, applications, identification and determining of aldehydes or ketones, carbonyl or amino compounds purification, or protecting the groups throughout complex or sensitive reacting.[3] They correspondingly create elementary units in specified dyes. SBs usually stand for bi-or tri- dentate ligands able to produce highly stable complexes with transitional metals [4]. Several of them can be employed as liquid crystals. SB reactions have been advantageous in creating carbon-nitrogen bonds in organic synthesis [5]. SBs act as an imperative intermediary in a quantity of enzymatic reacting containing interacted enzyme with the amino or a carbonyl collection of the substrate. The most essential categories of catalytic process is the biochemical activity that includes condensed primary amine in the enzyme with usual lysine residue and carbonyl cluster of the substrate for forming an imine and SB.[6] Stereo chemical analysis based on molecular model has depicted that the formed SB concerning methylglyoxal as well as amino group of lysine side chains of proteins can be headed for number of atom of peptide groups in which the charge transfer can arise among oxygen atoms of SBs and these groups .[7] The pyridoxal SBs resulting from amino and pyridoxal acids had made and examined based on biological viewpoint. Enzyme models based on transition metal complexes of these ligands are very imperative in biochemical scopes.[8] The speedy expansion of these ligands have caused enhanced research activity for coordination chemistry with highly remarkable deductions.[9] In the current paper, we focusses on the formation and biological actions of several primary amines derivative SBs as well as its complexes.

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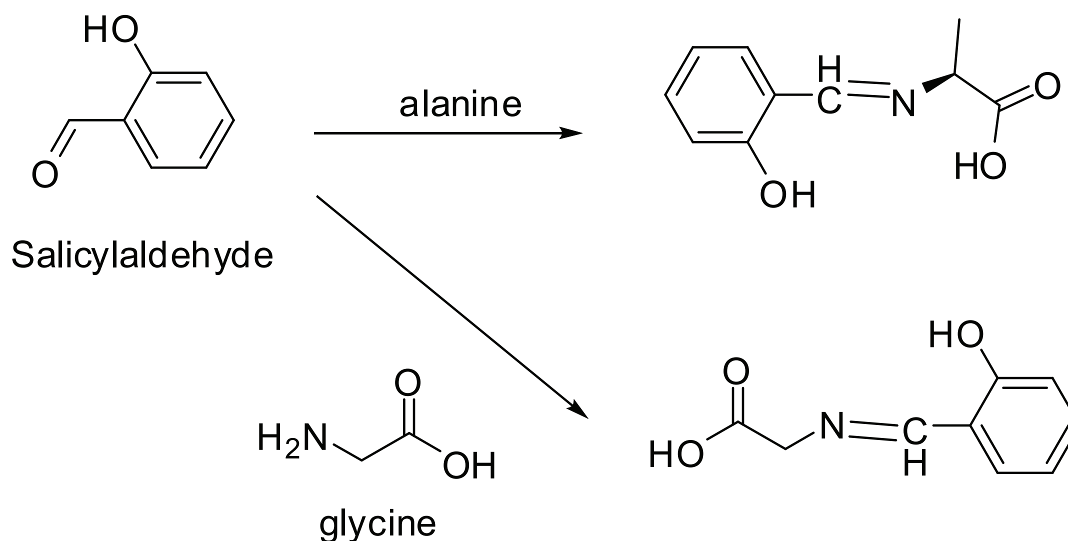
Part one: SB of Amino Acid

Metal chelates of prepared SB as a result of uniting salicylaldehyde along with amino acids are effectually employed in realizing the process of transamination reacting. These can be biomimetic species. The present research paper is concentrated on the characterization, complexation , syntheses, behaviour and antimicrobial analyses of several formed new SBs from salicylaldehyde with 3-amino benzoic acid besides Glycine and Alanine

employing sodium hydroxide as a catalyst. The formed SB ligands were efficaciously complexed with Zn (II) metal and examined based on their spectral data. Morphological analyses have implemented by means of SEM. The complexation impact on the antimicrobial action of SBs besides its Zn (II) complexes is as well investigated.[10]



Scheme1- Preparation of amino acid based Schiff bases

In platinum (II) complexes having lessened amino acid ester SBs have interacting with salmon sperm DNA has been examined by dint of circular dichroism and ultraviolet spectroscopies. The potential antitumor performance of each compound has been *investigated in vitro* on HeLa and A549 tumor cell lines. Practically, each complex had superior cytotoxic action as compared to cisplatin in contradiction of these cell lines. [11]

Synthesizing new SB compound can be resulting from s-allyl cystiene and methionine. A new formed compound, 2-((2-((2-(allylthio)-1-carboxyethyl) imino)ethylidene)amino)-4-(methylthio)butanoic acid (ACEMB) has been categorized. ACEMB has shown potent *in vitro* antioxidant[12].

On the other hand, 3 integrated ligand Cu(II) complexes [Cu(o-vanillin-L-tryptophan Schiff base)(diimine)] (diimine =2,2′-bipyridine (1), 1,10-phenanthroline (2) in addition to 5,6-dimethyl-1,10-phenanthroline(3)) have been formed and categorized based on spectral and analytical approaches. In the gas phase for B3LYP/LanL2DZ levels, the 1–3 molecular structures have been boosted by means of density functional theory (DFT). [13]

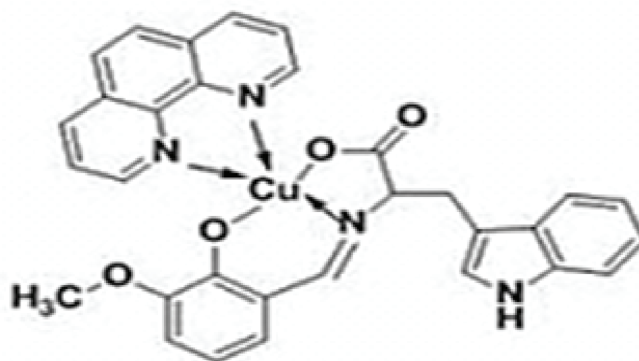
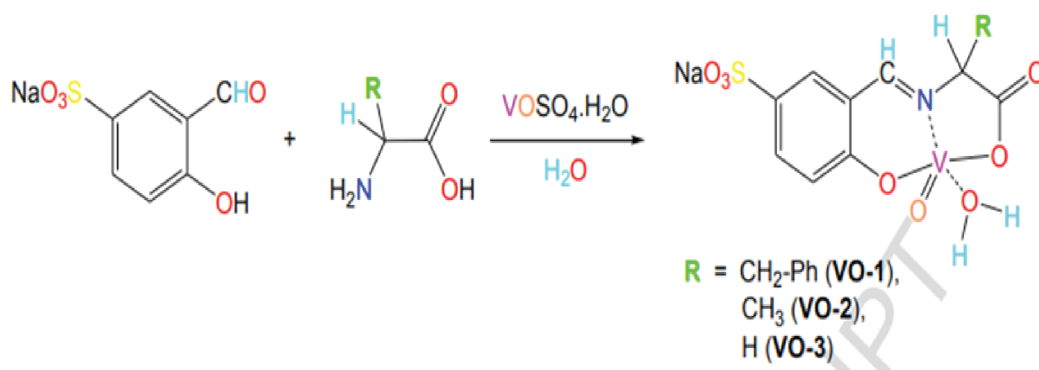


Figure 1- structure of cu complexes

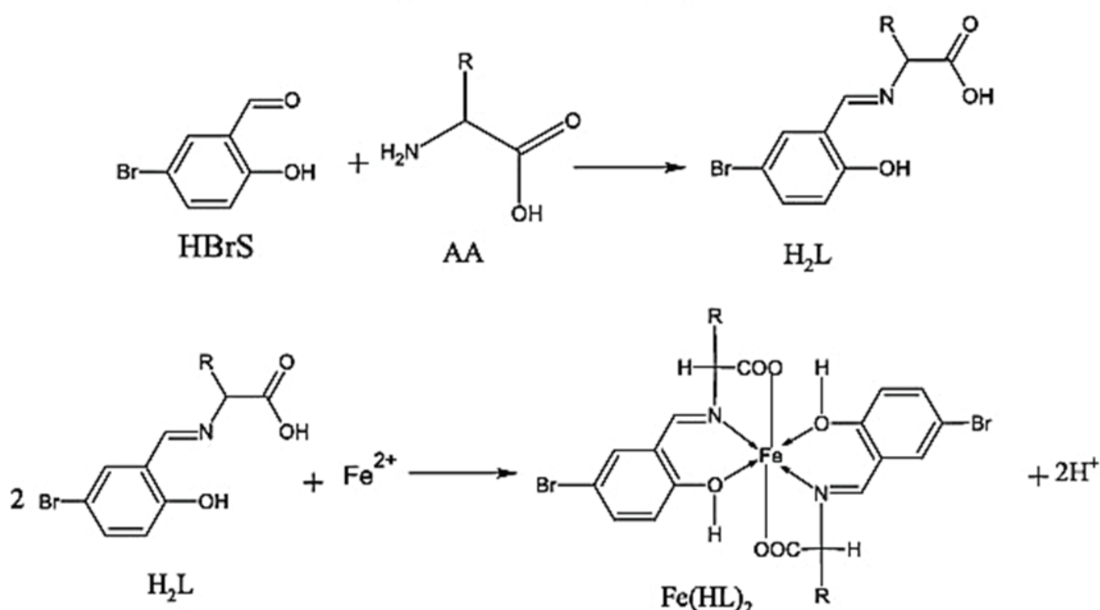
Critical investigation of thirteen crystallographic configurations of several Ni(II) complexes of amino acid SBs has performed. The foremost conclusion of [14] ha been the consequence of a parallel displaced category of aromatic interacting among Pro *N*-benzyl rings and *o*-amino-benzophenone. The aromatic interactions quality has revealed to be as controlled steric environment nearby amino acid side-chain, interpreting substituted Ni(II) complexes under dissimilar thermodynamic stabilities. [14]

3-anionic oxide-vanadium SB *N*-salicylidene amino acid complexes have been resulting by reacting sodium salicylaldehyde-5-sulfonate with phenylalanine (VO^{-1}), alanine (VO^{-2}) or glycine (VO^{-3}), subsequently, and mixed with vanadyl sulfate monohydrate. Inhibiting consequence of VO^{-3}, VO^{-2}, in addition to VO^{-1} has examined for carbon steel corrosion in chloride acid solution. Based on electrochemical experiments and in an existence of 1.0 mm VO^{-1} inhibitor, VO^{-}complexes preserve carbon steel corrosion, producing extreme inhibiting efficiency equal to 94.7%. Each VO-complex has mixed-type inhibitors.[15]



Scheme 5- The synthetic of the VO salicylediene amino acids complexes

By condensing 5-bromo-2-hydroxybenzaldehyde along with α-amino acids (L-alanine (ala), L-phenylalanine (phala), L-aspartic acid (aspa), L-histidine (his) and L-arginine (arg)), Novel chains of Fe(II) complexes and SB amino acids were proposed and formed by bioactive ligands. Infrared, ultraviolet–visible spectra, elemental analyses,, in addition to magnetic susceptibility and conductivity measurements have been employed to clarify the geometry of anew primed Fe(II) complexes. Additionally, the stability along with stoichiometry coefficients of the organized complexes was spectrophotometrically measured. The consequences put forward that the organized SB amino acid ligands perform as dibasic tridentate ONO ligands and have binding to Iron(II) in octahedral structure as stated by the typical formulary $[Fe(bs{:}aa)_2].nH_2O$. In the case of pH = 7.2 and based on electronic absorption spectra besides viscosity measurements, DNA interacting of these complexes has been examined. The investigational consequences specified that the examined complexes can be binded to DNA by means of intercalative mode and have shown a dissimilar DNA binding action based on this arrangement: bsari > bshi > bsali > bsasi > bsphali. Besides, the organized compounds have investigated for their *in vitro* antibacterial action in contradiction of 3 kinds of bacteria, *Bacillus cereus, Pseudomonas aeruginosa* as well as *Escherichia coli*. The consequences have given an indication that the metallic complexes have been in a greater reactivity concerning their resultant SB ligands [16].



Scheme 6- The formation of the investigated Schiff base amino acid

Ligands and their complexes

Synthesized novel metal complex of Co and Zn withamino acid-nucleobase hybrid ligand were accomplished through minimal chemical reacting of metal salt with amino acid L-histidine in addition to nucleobase adenine as ligands. The reactivity concerning BSA has shown that BSA fluorescence quenching by the dual complexes have been in static quenching, while as compared with the complex 2, complex 1 has the greater BSA-binding capability. The pharmacological action of the ligand in addition to the complexes has examined through antioxidant activity. They display implying influence in contradiction of radical DPPH. The consequences of the molecular docking investigations of reinforced thecomplexes.(17)

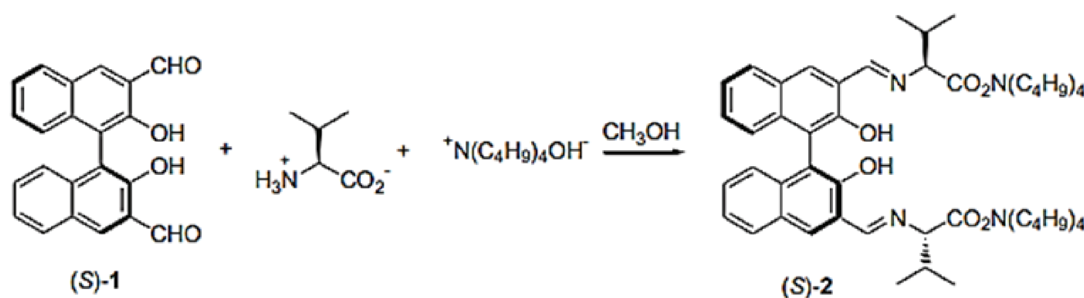
For amino acid SBs, formation and organizational categorization of cobalt (II) complexes has primed based on Salicylaldehyde and 3 amino acid in basic medium (Leucine, Valine , and Isoleucine) . The SBs and their metallic complexes have been initially scanned in contradiction of numerous microbes strains to investigate their biological influence. (18)

Expansion of innovative drugs stands for important solution to oppose in effect antimicrobial resistance threat. SB and Cinnamaldehyde-amino acid compounds stand for anew detected compounds that show noble antibacterial action in contradiction of gram-negative and gram-positive bacteria. Quantitative structure–activity relationship (QSAR) approach has employed for exploring the relationship among compound structures and antibacterial activity. (19)

Amino acid derivative SB as formed by reacting leucine with salicyldehyde in basic medium. A SB has employed as a ligand that reacted with Mn, Co, Cd and Cu metals with the intention of forming stable complexes. Each compound with ligand and complexes have correspondingly involved with diverse bacterial (staphylococcus aureus, Bacillus subbtilis and Escheria coli) and fungous strains (Aspergillus flavus, Aspergillus niger and Alternaria alternate) with the aim of investigating inhibiting activities for titled compounds. The consequences have depicted that the metallic complexes possess larger antimicrobial actions as compared with ligand. (20)

The amino-acid for SB (*S*)-**2** has organized based on condensing (*S*)-3,3′-diformyl BINOL (BINOL = 1,1′-bi-2-naphthol) with L-valine in an existence of tetrabutylammonium hydroxide in methanol. The compound shows off-on-off fluorescence reaction concerning Zn^{2+}. Spectroscopic investigations expose

(*S*)-**2**reacts with 1 equiv Zn^{2+} for forming the dimeric [2+2] complex with significantly improved fluorescence. Extra quantity of Zn^{2+} feasibly produces dissociated dimeric complex for giving significant diminished fluorescence. [21]



Scheme 10- Condensation of Schiff Base (S)-2

Resulting from 2-hydroxynaphthaldehyde with glycine and phenylalanine, Zinc (II), Cu (II), nickel (II), cobalt (II) and Iron (III) complexes of SB (LG, LP) have been testified. The LP, LG and their metallic complexes were partitioned for their antimicrobial actions in contradiction of 5 Gram-positive (Methicillin resistant Staphylococcus aureus (MRSA), Staphylococcus aureus, Streptococcus mutans, Enterococcus faecalis as well as Bacillus cereus) and 3 Gram-negative (Klebsiella pneumonia, Pseudomonas aeruginosa besides Escherichia coli) along with Candida albicans via broth microdilution methods. Resultant data have shown that ligands along with their metallic complexes have shown medium to good actions in contradiction of fungi and Grampositive bacteria. [22]

Scheme 11- The general synthesis procedure of ligands and complexes

The SB resulting from condensing tyrosine along with salicylaldehyde has been produced. In addition, its Pd(II), Pt(II) and Cu(II) complexes have primed. Antibacterial performance for the complexes has determined in vitro in contradiction of diverse bacterial strains as in Bacillus subtilis along with Pseudomonas aeruginosa using well-diffusion technique. Transition metal complexes possess noteworthy developed antibacterial activity as compared with parental drug. [23]

Part 2: mixed ligand of several amino acids

The ternary complexes of Zn(II), Cu(II), Cd(II) along with Ni(II) with 2-aminomethylbenzimidazole (AMBI) and glycine. The secluded chelates were partitioned for their antibacterial and antifungal actions using disc diffusion technique. The compound cytotoxicity in contradiction of larynx (HEP2) and colon (HCT116) cancer cells thoroughly investigated. For ternary M-AMBI-Gly complexes, stability constants have evaluated in aqueous solution at I = 0.1 mol dm3 NaCl potentiometrically. [24]

The Ni(II) , Co (II), Zn(II) , Cu(II), Hg(II) and Cd(II) complexes of integrated amino acid (L-Proline) as well as Trimethoprim antibiotic have formed. The ligands and their metal complexeswere have for their antimicrobial performance in contradiction of 4 bacteria including gram positive besides gram negative types. [25]

Diverse ligand complexes of bivalent metal ions with Ni(II), Co(II), Zn(II), Cu(II), Cd (II), and Hg(II) for [M(Anth)2(TMP)] composition in 1:2:1 molar ratio, in which AnthrH= Anthranilic acid (C7H7NO2) and

Trimethoprime (TMP) = (C14H18N4O3) were formed and categorized. The dual ligands along with their metallic complexes were screened for their bacterial performance in contradiction of particular microbial strains gram positive and gram negative types. [26]

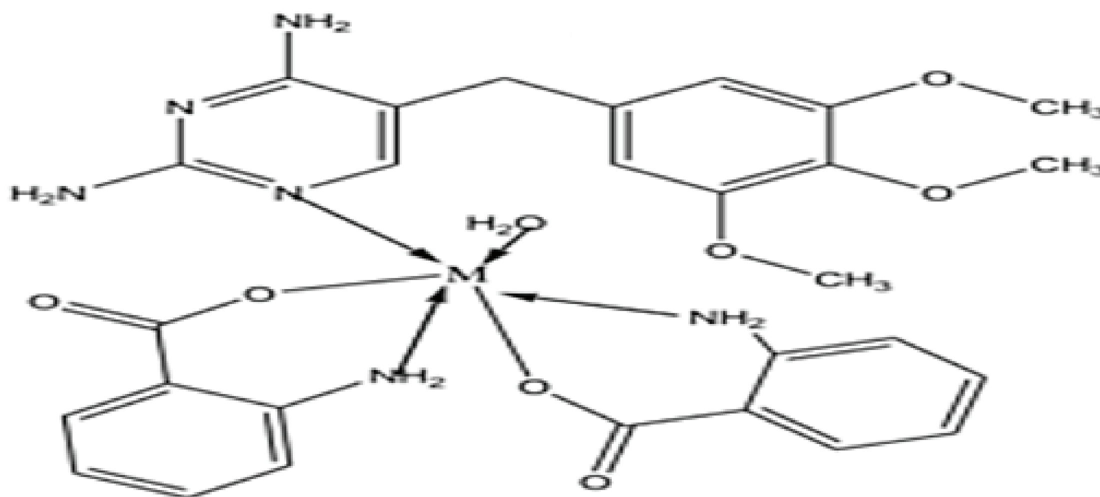


Figure5-preparation of the Complexes [M(Anth)2(TMP)]

Through condensing {3-amino-1-phenyl-2-pyrazolin-5-one (APO)} and o-phathalaldehyde and anthranillic acid (L2) accompanied by several transition metallic ions, mixed ligand complexes with SB derivative ligand (L1) were created. All these compounds were determined in contradiction of mirobial strains gram positive and gram negative types based on the analytical studies.[27]

A different mixed Iron (III) and Zinc (II) complexes along with iso nitroso acetophenone (HINAP) besides L-amino acids like, L-histidine, L-proline and L-phenylalanine, were formed and characterized. The antimicrobial performance has examined in contradiction of bacteria (bacillus subtilis, Staphylococcus aureus,, *Pseudomonas aeruginosa*, *Escherichia coli)* and *Candida albicans* fungi . The Fe and Zn complexes have been in a higher activity active in contradiction of Gram + than Gram - bacteria. They likewise exhibit significant growth inhibiting in contradiction of the tested fungi. In vitro antitumor activity has examined in contradiction of cancer cell lines of larynx or HEP2 type has shown significant ligands toxicity with their mixed complexes. [28]

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

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

FundinG: Self-funding

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Measuring of Interleukin -22 and IL -17a Levels in Seropositive and Seronegative Rheumatoid Arthritis Patients

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Abstract

Background: "Rheumatoid arthritis (RA) is an inflammatory disease leading to joint disruption". The instant study designed to detect serum markers in RA that could discriminate between seropositive (SP) and seronegative (SN) by evaluating levels of IL- 22and IL -17a in patients with rheumatoid arthritis. ***Methods***: In this cross-sectional study, a total of sixty Rheumatoid arthritis (RA) patients' age and sex-matched with healthy controls were involved in the present study. The serum IL- -22 and IL -17a levels were measured using an ELISA kit. Results: The mean ±SD age in seropositive and seronegative was (37.22± 11.29 and 34.28±20.3 years, respectively), while in control group was (27.14±9.33 years). Furthermore, serum IL- 22 and IL -17a level was significantly higher in seropositive and seronegative RA patients compared to healthy controls (P<0.001). There was no significant variation in serum IL -22 and IL -17a level according to the seropositive and seronegative in RA patients (p>0.05), but there was a positive correlation between them ROC test representing a highly sensitive and specific . **Conclusion:** The present study exhibited higher serum IL- 22 and IL -17a levels in seropositive and seronegative in RA patients compared to healthy controls. Therefore, IL- 22 and IL -17a can be considered as a biomarker for RA disease, with high sensitivity and specificity but these cytokines couldn't be used as discriminated between SP and SN in RA patients.

Keywords: *- Rheumatoid arthritis, Seropositive, Seronegative IL22, and IL17a*

Introduction

"Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation of the synovial membrane [(1)] Multiple studies have been demonstrated that levels of disease-related biomarkers may be elevated prior to the onset of symptomatic rheumatoid arthritis [(2),(3)] . These biomarkers include rheumatoid «factor (RF), antibodies to «citrullinated protein antigens (CCP), as well as multiple cytokines/chemokines», C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) [(4), (5).] Releasing "pro-inflammatory cytokines, as well as other pro-inflammatory molecules, reaches to the mediated of cellular differentiation, inflammation, immune pathology, and regulation of the immune response [(6)]. Selectively Th17 cells generate the signature cytokines such as interleukin 17 (IL-17), IL-21 and IL-22, and have been reported to play a vital role in the chronic inflammatory response and affected consequently joints and led to tissue damage in RA patients [(7)]. The interleukin (IL-22(was a member of the IL-10 family that significantly controls tissue responses to inflammation 2". A few studies propose the pro-inflammatory/pathogenic role of IL-22 in the onset and progress of RA [(8)]. "The object of this research was to assess the role of certain disease markers such as autoantibodies (ACCP, RF), "cytokines (IL-17 and IL-22) and (hs-CRP, ESR) in the pathogenesis of RA as panel useful in assessing disease with seronegative and seropositive besides efforts to search whether of these cytokines could distinguished between SP and SN included in the of RA .

Subjects, Materials and Methods

"The considered investigation included 60 patients

with RA (50 females and 10 males), including new diagnoses without treatment divided to 36 seropositive, 24 seronegative providing the "American College of Rheumatology (ACR) criteria for RA". These RA patients were diagnosed by the clinic's rheumatologists and participation of the "Rheumatology Department / Baghdad Teaching Hospital" within the period of Feb 2019 to June 2019. Each Rheumatoid arthritis patients were chosen after excluding patients with any of the following states such as "malignancy, other autoimmune diseases, pregnancy, medications including steroids, antibiotics, CNS" depressants, hormonal therapy, and others. Unrelated, fifty healthy individuals were correspondent to the patient's group in their ages and genders were randomly selected as controls. During the morning five milliliter of Blood was collected from all members of the study groups. Each sample was separated into 2 parts; the first part is the serum for the serological tests, while the second is whole blood for ESR. The concentrations of the serum IL-22 and IL-17 levels in patients and healthy controls were determined by using ELISA Kit, according to the manufacturer's guidance (MyBiosource .USA). On the other hand, RF and ACCP measured by ELISA (Chorus, Italy) the highly sensitive C –reactive protein (hs-CRP) were detected by ELISA (Demedltec diagnostics) and ESR was tested by Westergren method

Statistical Analysis

Statistical analyses were conducted using the SPSS statistical package for Social Sciences (version 20.0 for Windows, SPSS, Chicago, IL, USA). Data are displayed as mean ± SD for quantitative variables.While number and percentage for qualitative variables. Quantitative data were tested using ANOVA and Kruskal-Wallis test for differences between groups, Pearson's correlation for the relation between groups; while qualitative relations were evaluated using the Chi-square test. P-value of <0.05 was considered statistically significant. Cut-off values were estimated according to ROC [9].

Result

Demographic characteristics of the studied groups:

Table 1 shows the baseline characteristics of the studied samples according to age and gender with a comparison of significance in studied groups shows that a total of 100 individuals were divided into two groups 60 patients with RA [35 seropositive (SP) and 25 seronegative (SN)], while 40 individual chosen as apparently healthy controls. Results in this table demonstrated that there was no statistically significant difference between males and females in RA patients and control at P>0.05. Moreover, no significant difference was reported in the current study according to age groups between RA groups and control at P>0.05.

Table 1: The baseline characteristics of the studied groups.

Variables		RA Patients		Control	P-value
		seropositive	Seronegative		
Age (years)	Range	(25-64)	(20-61)	(20-50)	P=0.677 NS
	Mean ± SD	37.22± 11.29	34.28±20.3	27.14±9.33	
Gender	Male No. (%)	7 (20 %)	6 (24 %)	20(50%)	P=0.955 NS
	Female No. (%)	28 (80 %)	19 (76 %)	20(50%)	
Total No.		35	25	40	

(*) NS: Non -Significant. at P>0.05.

The concentration levels of ESR, RF, hs-CRP, and ACPA among studied groups

Table 2 representing the Mean± SD of ESR, RF, hs-CRP, and ACPA, according to studied groups. Analysis study shows that there were no significant differences in the Mean± SD between seropositive (76±23.5;

16.43±9.98, respectively) and seronegative(68±86.2; 17.76±10.29, respectively) in RA patients according to ESR, hs-CRP at P>0.05. Hence, that there was a significant difference in the Mean± SD of RF, and ACPA in RA patients between seropositive (372.5± 34.7; 22.6± 11.6.9, respectively) and seronegetive (12.8± -8.7; 6.6± 1.9, respectively) at P> 0.001. While, the mean concentrations of «ESR, RF, hs-CRP and ACPA» in seropositive RA patients were significantly higher than healthy control (P < 0.001).

Table 2: distribution of serological and blood marker in the studied groups.

Parameters	**Patient seropositive**	**Patients seronegatie**	**Control**	**P-value**
ESR mm/h M ± SD	7 6± 23.5	68 ± 36.2	1 6± 2.5	0.000
hs-CRP mg/L M ± SD	16.43 ± 9.98	17.7 6± 10.29	3.47 ± 10.65	0.000
Anti-CCP AU/ml M ± SD	22.6± 11.6.9	6.6± 1.9	5.7± 7.2	0.000
RF AU/ml M ± SD	372.5± 34.7	12.8± -8.7	11.8± 6.7	0.000

Highly Sig. P=0.000 among patients groups and healthy control

Mean levels of IL-22 and IL-17a among the studied groups

The mean concentration of serum IL-22 level was significantly higher in RA patients (SP and SN) than healthy control. The same was true for serum IL-17a level exhibited a significantly higher mean concentration in RA patients versus control P<0.000) .Notably that no differences in mean concentration of both IL-22 and IL-17 between RA patient (SP and SN) as showing in table 3.

Table 3: Mean concentration of serum cytokines IL-22 and IL-17a among studied groups

Parameters	RA Patient seropositive	RA Patients seronegatie	Control	P-value
IL-22 pg/ml M ± SD	26.6 ± 38.7	21.2 ± 27.5	7.4 ± 8.0	0.000
IL-17a pg/ml M ± SD	18.4 ± 15	19.8 ± 11.2	9.6 ± 6.7	0.000

Highly Sig. at P=0.001 among patients groups and healthy control

Pearson's Correlation Coefficients between IL-22 & IL-17a among RA seropositive

In the current study, there were significant correlations between IL-22 with IL-17a (r=0.353 with P=0.032), in seropositive RA patients and (r=0.297 with P=0.024) in seronegative as show in table 4.

Table 4 : Pearson's Correlation Coefficients between IL-22 & IL-17a among RA groups

Groups	Studied parameter	Pc &p-value	IL-22	IL-17a
Seropositive RA Patients	IL-22	r		0.353*
		p-value		0.042 (S)
Seronegative RA Patients	IL-17a	r	0. 297*	
		p-value	0.024 (S)	

Pearson Correlation. S.: significant *: Significant at Ps values (0.05)

Estimation of cut-off values, ROC curve, sensitivity and specificity of the IL-22 &IL-17a among RA groups:

In RA patients , the IL-22 cuts-off values was 27.6 pg/ml with sensitivity 86.8 % , specificity 93.9%, and the AUCROCs of s0.952 (P = 0.000) ; while in IL-17a cuts-off values was 16.24 pg/ml with sensitivity 93.4% ,specificity 99 %, with AUROCs of 0.934 (P = 0.000). Table 5

Table 5 : Estimation of cutoff points, sensitivity, specificity and AUROC of the parameters in RA patients .

Parameters	Cut-off points	Sensitivity (%)	Specificity (%)	AURO	P-values
IL-22 pg/ml	27.6	86.8	93.9	0.952	0.000
IL-17 a pg/ml	16.24	93.4	99	0.934	0.000

AUCROC: Area Under receivers operating characteristic, , Sig. at P value < 0.05.

Discussion

Rheumatoid arthritis (RA) refers to systemic chronic inflammatory disease leading to joint destruction. [10]. information on the differentiation in the systemic inflammatory serum markers between SP and SN RA is insufficient .Consequently, in the current study preferred of serum markers were evaluated in self-governing groups of SP and SN RA patients. It has been suggested that different inflammatory pathways are involved in the development of seronegative (SN RA) and seropositive RA (SP RA). The existing study exposed that female patients were nearly three times more predisposed to RA than men this result was agreeable to some extent among that of local prior studies in Iraq [11], [12]. The preponderance of particular disease activity markers representing the appearance of autoantibodies in early RA these including ACCP and RF that have been conferred a higher risk of further aggressive and prognostic markers of an erosive disease compared to the population had negative for both autoantibodies [13]. The immune complexes containing –ACPA established to induced production of pro-inflammatory cytokines and triggering of macrophages via Fcγ R-dependent augmented by the presence of IgM RF in this process [14]. Regarding ESR and reactivity of h-CRP, it was noted that ESR, significantly raised in seropositive (SP) and seronegative (SN) RA patients, which is nearly harmonious with results of previous works of literature [15],[16] .Here rise in the seropositivity of active disease markers has been additionally connected with continued active inflammatory registered by the long-term height of erythrocyte sedimentation rate (ESR) and disease activity score [17]. The highly-sensitivit CRP (hs-CRP) assay can be applied to distinguish mild disease activity that was associated with inflammation but that was not detectable by routine CRP testing [18]. The results of hs-CRP in this study correspond with other studies [19], [20]. Those studies were proved that hs-CRP consider as a cardiovascular (CV) disease brand in RA patients plus dropped a spot of light on the high incidence of patients with or at the hazard of progressing to CV diseases in RA. Aforementioned study result of increased IL-17a level in RA patients would coincide with many other studies [21],[22], and established its important role in RA pathogenesis . Previous studies found the concentrations level of IL-17a in both serum and synovial fluid were greater in patients with RA than in healthy and recommend that high IL-17a levels may be correlated with a more severe clinical development as mentioned by [23],[24]. Meanwhile, another study observed strong associations of serum and synovial fluid IL-17a levels found with ESR, CRP, RF, and anti-CCP [25], those findings imply that raised serum and synovial IL-17a levels in RA patients correspond to the development of the disease activity and severity. Besides, Metawi *et al* observed that levels of IL-17a in serum and synovial fluid revealed significant positive associations with Disease Activity Score in 28 joints (DAS28) in a cohort of 30 patients with active RA with knee diffusions, while the worse functional state was associated with greater IL-17a levels [26]. In contradiction with the study on RA Egyptian patients, reported that serum level of IL-17a didn't correlate with the DAS28 score but it was significantly greater among critically active patients as corresponded to patients with modest activity [27].

conclusion: There was an elevation in both IL-17 and IL-22 in seropositive and seronegative RA patients with high sensitivity and specificity that means IL-22 and IL-17 would act as pro-inflammatory cytokine during the disease course of RA but they couldn't use for discriminated between SP and SN RA.

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

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

Funding: Self-funding

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Detection of *B1 Gene* in *Toxoplasma Gondii* and the Role of Interleukin 2 in Abortive Women Infected by this Parasite

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Abstract

The study included 80 blood and serum samples of aborted women with *Toxoplasma gondii* infection and healthy, uninfected and non-aborted women in the control group. For the period from August-2018 to June-2019, in collaboration with the Falluja teaching hospital for women and children and the Ramadi teaching hospital for women and children, the Enzyme-Linked Immunosorbent Assay (ELISA), also to the *Toxoplasma gondii (B1) gene*, the Nested polymerase chain reaction technique are used. The results of the study showed by that Elisa *Toxoplasma gondii*, IgG infection (50%), IgM infection (12.5%), and IgG & IgM Infection (12.5%), were mean (108.16±47.02) (99.02±31.47) (96.45±20.68) respectively compared with the control group (25%) (0.94±0.93) and Interleukin-2 shown in IgG group (48.66±12.01) and control group (14.28±6.84) decreased compared to IgM (81.19±9.66), and IgG & IgM (80.71±9.18) group. Nonetheless, using nested PCR, the *B1 gene* showed just (12.5%) of all study samples in IgM and IgG&IgM alone and all IgG negativly.

Keywords: *Toxoplasma gondii, ELISA, Interleukin 2, nPCR.*

Introduction

Toxoplasmosis is the main common parasitic disease, in which the coccidian protozoan caused by *Toxoplasma gondii,* (from 10% to 80%) in different parts of the world. The human infection pathway is the consumption of tissue cysts found into uncooked or raw eating as well as the ingestion of oocysts through polluted vegetables or water, another path of infection is the transmitting of tachyzoites (the fast-dividing) from mother which pregnant to the growing fetus which can be resulting in abortion, chorioretinitis, including severe developmental defects **[1].**

In *T. gondii*, it was found that three different phases of the life cycle depend on the phase of the parasite into the host. Tachyzoites and bradyzoites found mainly in the intermediate host (asexuality, within the tissue cyst) appear to be a morphological form. The most recent phase of sporozoites can only be seen in the most recent host (sexuality reproduction, oocysts) **[2].**

The most significant sequel to *toxoplasmosis* is a congenital infection in women pregnant [3]. The congenital transmission of *T. gondii* happens predominantly for the first time during pregnancy [4]. The incidence of congenital toxoplasmosis is greatest for the first and second trimesters of pregnancy, usually leading to abortion or stillbirth **[5].**

Many serological methods have been used to detect *T. gondii* as an enzyme-linked immunosorbent assay. Recent research has confirmed that PCR is critical to assessing the prevalence of *T. gondii*, as the only method of DNA detection, PCR has been used professionally in women pregnant to identify *toxoplasmosis* **[6]**. The *B1 gene* appears highly specialized and has 35 replicates in its genome, resulting in the amplification of the target for polymerase chain reaction (PCR) to identify blood and tissue parasites **[7].**

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Interleukin-2 (IL-2) is a very considerable inflammatory cytokine, with IL-2 production abnormalities identified in patients receiving transplant rejection. A number of studies have shown that the higher IL-2 outflow levels have already been an association with recurrent spontaneous abortion [8].

Material and Method

Collection of samples

Between August-2018 and June-2019, in collaboration with Falluja teaching hospital for women and children, Ramadi teaching hospital for women and children, 80 blood samples were collected over the age range (18-45) years. In this study, four different women's groups, control group included (20 samples) for healthy women non-abortion before, but infected women with *T. gondii* IgG group (40 samples), IgM group (10 samples) and IgG&IgM group (10 samples) which had an abortion before on the basis of the first diagnosis.

5 ml of venous blood samples were collected from the patient and the control subjects, 3ml was placed in a gel tube (without anticoagulant) and kept at room temperature until blood was clotted; the serum was separated by centrifugation at 3500 r.p.m. for 10 min and then stored at -20 ˚C. Also 2 ml placed in the EDTA tube and stored at-20 ° C until DNA was extracted. The DNA extraction by Kit extraction Geneaid from the blood as well as stored at-20 ° C until used for PCR.

The Diagnostic Methods:

1-Serological tests

A. Estimation antibodies of *Toxoplasma gondii*

The serological test was used for the diagnosis of *Toxoplasma gondii* by the ELISA test IgG according to the NO.TOXG01 catalog kit and IgM according to the NO.TOXG02 catalog kit.

B. Estimation of IL-2 concentration

Estimation of the level of IL-2 in the serum of women with T. Gondii was done using the ELISA method, according to the use of the Cloud-Clone Crop Catalog kit NO. SEA073Rb.

2- Molecular detection test

Nested Polymerase chain reaction (nPCR)

Blood DNA extraction was carried out according to the DNA extraction kit protocol with NO catalog. Nested PCR performed a two-stage amplification of the fragment of the B1 gene with separate primer pairs shown in table (1) for all DNA samples.

Table (1): *Gene B1* Specific Primers

B1 gene Primers	*B1*gene fragment	Fragment Length(bp)	Annealing temperature Tm
B1 (F1) 5′-GGAACTGCATCCGTTCATGAG-3′	694-714	193 bp	56.6 ºc
B1 (R1) 5′-TCTTTAAAGCGTTCGTGGTC-3′	887-868		51.9 ºc
B1 (R2) 5′-TGCATAGGTTGCAGTCACTG-3′	757-776	96 bp	51.7 ºc
B1 (F2) 5′-GGCGACCAATCTGCGAATACA CC-3′	853-831		63.2 ºc

* F: Forward sequences, R: Reverse sequences.

Primers were added in the first stage, as instructed by the manufacturer, and are then placed into the PCR, then the first stage product is used as a template in the second stage and is also put into the PCR thermocycler.

Result and Discussion

Serological tests are beneficial for the diagnosis of T. gondii by detecting antibodies in serum and determining the phase of infection in the acute and chronic phase [9].

The result of this study shown in Table (2), *T.gondii* antibody level (UI / mL) in control group was (0.94±0.93) a significant difference with group IgG infection, IgM infection, and IgG & IgM Infection (108.16±47.02) (99.02±31.47) (96.45±20.68) respectively. But in group IgG had shown a non-significant difference with both groups IgM and IgG & IgM. Also group IgM had shown a non-significant difference in rates of *T.gondii* antibodies opposed to group IgG & IgM.

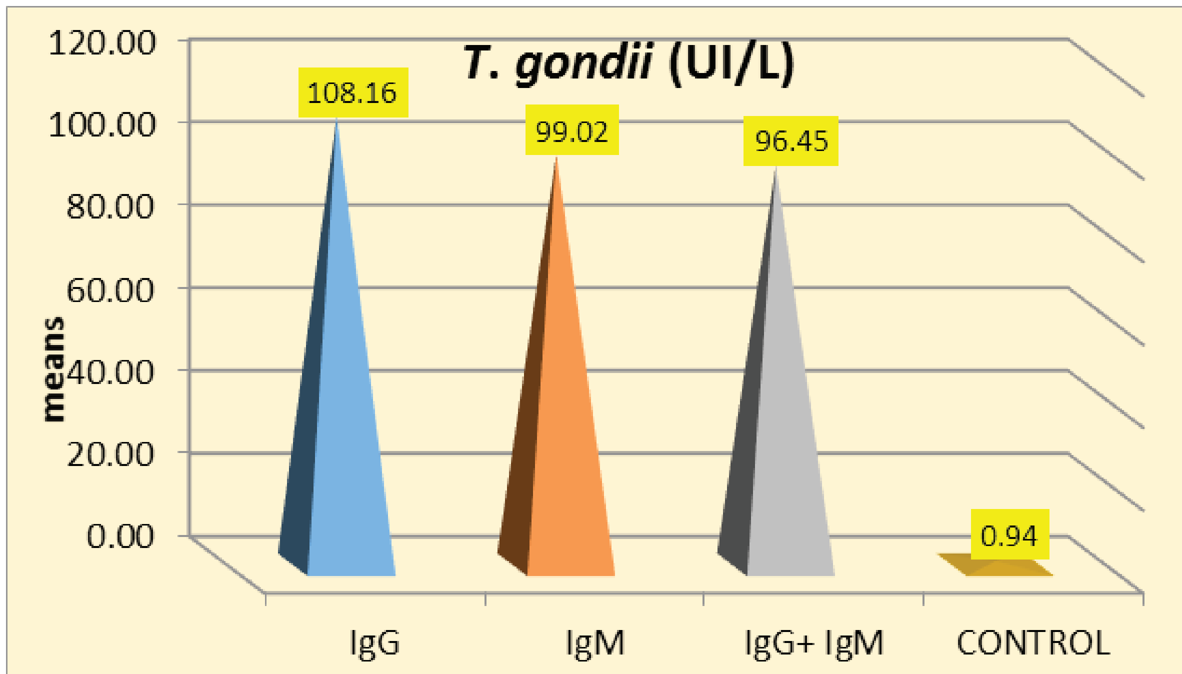

Figure (1): Levels of *mean T.gondii* infection in Patients and Control

The result of this study was shown to be a percentage of *T. gondii* seroprevalence IgG group (50 %) which is significantly greater than IgM and IgG&IgM which is shown (12.5 %) and (12.5 %) respectively, but control group (25 %), as most IgG-positive *toxoplasmosis* incidents were sufficient to determine the presence of chronic infection; IgM-positive titer, in the other hand, leads to the presence of acute infections.

The results of this study coincided with the results of the study [6] which showed *T. gondii* seroprevalence for IgM (4.16 %) and IgG (25.83 %) while IgM and IgG shown (3.33%) of aborted women with toxoplasmosis; and [10] were identified as IgG (31.5 %), IgM (7.6 %) and IgM & IgG (2.5 %).

On the other hand, the results were not in agreement with the result of [11] as shown IgG of *T. gondii* was (18.09 %) whereas IgM (9.79 %) for pregnant having recurrent spontaneous abortions also[12] observed 60 (38.7%) of IgG & IgM *T. gondii* were positive (4 % and 44 % respectively to IgG and IgM for aborted women).

Toxoplasmosis, which can lead abortion at any time of pregnancy, is indeed controversial, but even this study was been proposed to seek a possible association for both *T. gondii* infection and abortion.

The prevalence of parasite *T. gondii* varies among women in different countries in the world, based on different climate conditions, health, and food habits, economic standing, level of education and age [13].

The reason for high prevalence among aborted women may be reversion to the kind of acute infection or reactivation of chronic infection due to reduced immunity of pregnant mothers since the time of infection during pregnancy have an important role to play in determining the demise of the fetus [14].

Cytokines are divided into two components: Th2 (such as IL-3 and IL-15) to correct the immune system response, while TH1 (such as IL-2, IFR) seems to be harmful to end a pregnancy by influencing either the placenta or stimulation to avoid fetal implantation and to cause abortion or fetal killing.TH1 allows to invade trophozoites and apoptosis and hence generates harmful factors to the fetus [15].

In these study Interleukin-2 (IL-2) in women with *T. gondii* infection had a high level especially in comparison to the control group, which showed a lower level of infection. Furthermore, the increase in women infected with *T. gondii* IgM and IgG&IgM (81.19±9.66) (80.71±9.18) was higher than in the IgG (48.66±12.01) and control (14.28±6.84) respectively.

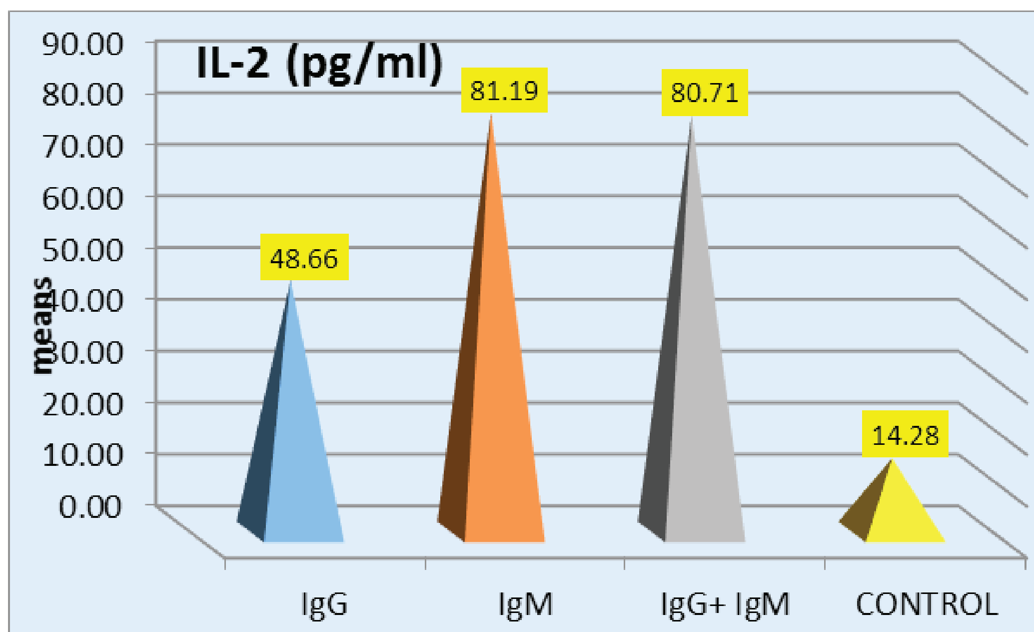

Figure (2): Levels of *mean* IL-2 in Patients and Control

These results have been similar to previous studies done by [16], [8] which found in a group of women diagnosed with *T. gondii*, the rate of IL-2 level was increased compared to healthy women as a control group.

IL-2 manages the activities essential for the immunity of white blood cells (particularly lymphocytes). It is part of the human body's normal response to microbial infection, besides being detectable in both nonself (foreign) and self. IL-2 effects have been mediated by CD4 and CD8 T-cell lymphocyte bind to IL-2 receptors of the immune response [17].

Also many unknown abortions are related to immunological reasons when cytokines mainly work on the loss or completion of pregnancy whereas Cytokines of kind Th1, such as (IL-2) are widespread in cases of pregnancy failure [18].

Following the initial ELISA serological examination, the nPCR technique is used to detect the existence of *T. gondii* DNA in circulating blood is an opportunity for an actual diagnosis in aborted women with *T. gondii* infection.

Results of n PCR in samples of patients (12.5%) of the DNA fragment band indicated an identification of the *B1 gene* and this explained the existence of the infection and had shown (92 %) that there is no amplified DNA fragment stating that there is no *B1 gene* and that there is may be, no infection.

The first step, of n PCR test has been used to amplification of *B1 gene* fragment (DNA) using the first primers to amplify the 193 bp fragment such as figure (3).

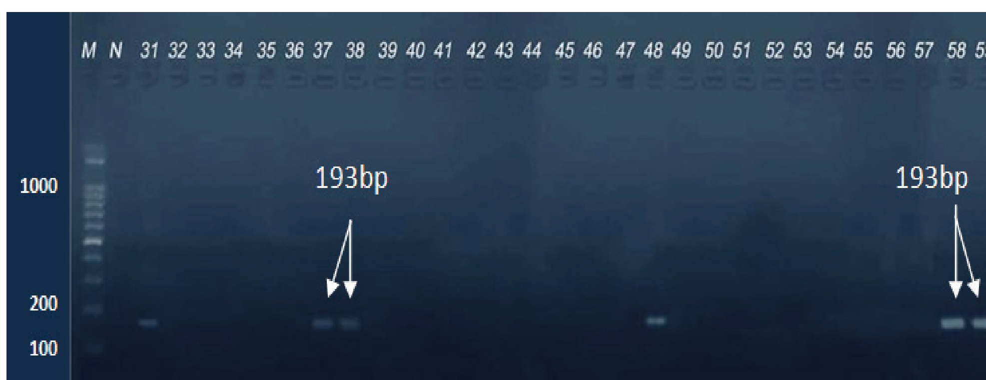

Figure (3): Amplification of the 193bp fragment of the *B1 gene* of *T. gondii* DNA

The second step, of the n PCR experiment has been used to amplification a 96bp fragment of the isolated DNA *B1 gene* from first product, that only in 10 samples (12.5%) for IgM and IgG&IgM showing positive and all other IgG negative being excluded such as figure (4).

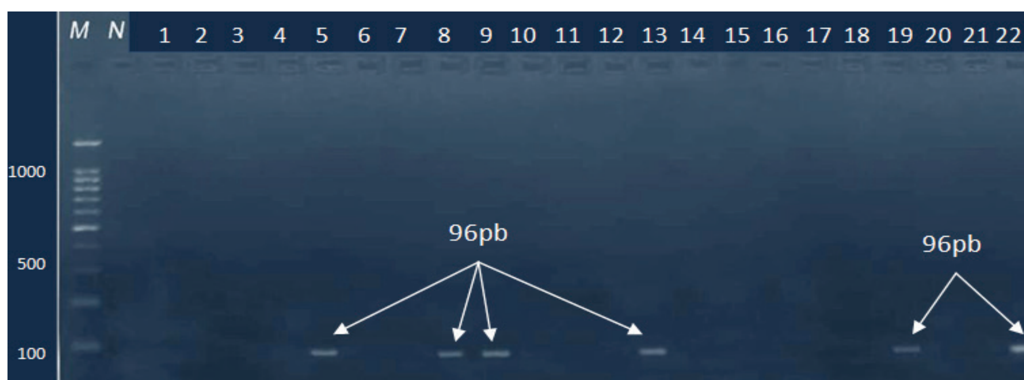

Figure (4): Amplification of the 96bp fragment of the *B1 gene* of *T. gondii* DNA from first product

These result may be due to IgM Ab can also be recognized in both acute and chronic *toxoplasmosis,* at that same time as *T. gondii* IgG Ab continued for even a long time, until for years. Therefore, the serology method may well be ineffective because it is focused on the level of antibodies generated which either fail or are delayed significantly whereas n PCR is based on the existence of the parasitic genetic material [(19)].

Also perhaps result due to the *B1* primers have the highest sensitivity due the two primers have been very specific to *T. gondii* strain was detected in Iraq. It is also very specific to the magnification DNA of *T. gondii* used in the PCR technique [(20)].

The result of the present study using n PCR were accepted with [(6)] as the result of the *B1 gene* was detected in (15.83 %) infected women with *T. gondii* and results of [(21)] also were consistent with the resulting study of investigation report *T. gondii* rate (31.57%) in donor blood. But, the results of [(22), (19)] were far from the result of these study *B1gene* was identified in (77 %) and (87.5 %) respectively of both the study samples.

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

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

Funding: Self-funding

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Bacteriocin Production in *Bacillus cereus* Food Isolates with Molecular Detection of *cerA* gene

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Abstract

Sixty-three *Bacillus cereus* strains isolated from food samples. All strains were subjected to DNA sequencing for 16S rRNA for identification. 15 strains were registered at GenBank of NCBI and given new accession numbers. 41.26% of the isolates showed bacteriocinogenicproduction activity against four bacterial speciesviz,*Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* sp. Presence of *cerA* gene coding for cericidine(a type of bacteriocin) was detected in 7.69% of the isolates that produced bacteriocin. Bacterionwas precipitated by ammonium sulphate and purified by dialysis. Antimicrobial activity of precipitated bacteriocin against the four types of bacteria showed the effect on *Bacillus cereus* and *Staphylococcus aureus* growth but not on *Escherichia coli* and *Salmonella* spp.The produced bacteriocin has a molecular weight ranging from 47-54 KDa.Estimation of the concentration and physical and chemical properties of bacteriocin were also investigated.

***Key word**: Bacteriocin, Bacillus cereus, cerA, 16SrRNA.*

Introduction

Bacteriocins are bacterial products which acts as antimicrobial peptides. They are ribosomally synthesized and secreted to act closely related to one another of bacterial species. Bacteriocins are used as antimicrobials for the treatment of human and animal infections. Such products will minimize the increased bacterial resistance to conventional antibiotics. In addition, since consumers require minimally processed foods without chemicals, natural antimicrobial research such as bacteriocin has increased[1]. Bacteriocins have an antimicrobial action affecting cell wall or cell membrane[2]. Bacteriocins are low molecular weight polypeptides with heat stable and proteolytic enzyme sensitivity[3,4]. Using of food additives has been reduced due to safety concerns. Chemical additives are sometimes replaced by the use of natural products of microflora using their antimicrobial activity to increase the lifespan and safety of foods[5,6].This study aims to isolation and purification of bacteriocin from *Bacillus cereus* and determination the molecular weight by SDS-Page. In addition to study its antimicrobial activity against several type of medically important bacteria.

Materials and Methods

Bacterial strains

Bacillus cereus strain was isolated from food samples from a previous study[7]. 63 of *B. cereus* strains were further identified by 16S rRNA partial sequence. Genomic DNA was extracted from bacterial cells cultured using a commercial kit provided by the manufacturer (Geneaid). Extracted DNA were stored at -20°C until used. The 16S rRNA oligonucleotide primers which were used have 1541 bp and their sequence are: forward, AGAATTTGATCCTGGCTTAG and reverse, AAGGAGGTGATCCAGCC[8].

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Screening of bacteriocin production in the isolates

Cross-streaking antimicrobial activity assay

Bacillus cereus strain was grown on Brain heart infusion agar supplemented with 1.5 % glucose. For testing the antimicrobial activity of*B. cereus*, strains were cross-streaked at 24 hrs pre-incubation. A sterile loop was streaked vertically in the center of BHIAG plate to form a single line, incubated at 35° C for 24 hour[9,10]. Four species of bacteria used such as *B. cereus, S. aureus, E. coli*, and *Salmonella* sp. The types of bacteria cultured horizontally on *B. cereus* from the edge to the center, after 24 hour of incubation, a clear of inhibition region was appeared, and this referred to bacteriocin production[11].

Molecular detection of *cerA* gene in *Bacillus cereus* isolates

The *cerA* gene was detected using theprimers with product size 233bp. The pair of primer used wasforward: ATGTCAAAAGGATACAAGTTCACAA and revers: TTATTTACAAATCTTAATTGACGT[12].The PCR tubes were transferred to the thermolcycler to start the amplification reaction according to the specific program.The PCRtemperature conditions were 94°C denaturation, 55°C for annealing step and 72°C for extension step. PCR products were detected in 1.5 % agarose gel stained with ethidium bromide and viewed by U.V. transilluminator.

Production of bacteriocin

The bacteriocin producing bacteria were grown in Brain-Heart Infusion broth (BHIB) supplemented with 1.5 % glucose[13]. After 18 hrs. incubation, the fermented broth was centrifuged at 8500 rpm for 20 mins[14].The supernatant was precipitated with 60% ammonium sulfate (w/v) and left tosettle overnight. To collect the precipitate, the product was cooled centrifuged at 5000 rpm for 45 min. The precipitate then dissolved in 10 mL of 0.1 M phosphate buffer (pH 6.0) and dialyzed against 500 mL of 0.1 M phosphate buffer (pH 6.0) at 4°C for overnight[15]. The produced pellets were centrifuged anddissolved inamount of water. 0.1 ml of the solution was tested for the antibacterial activity using well diffusion assay[14]. The protein then detected by biuret test using biuret reagent[17,18].

Estimation of extracted bacteriocin concentration

The concentration of extracted protein was estimated by using a spectrophotometer and a wavelength of 280 and 260 nm depending on the following equation[19];

Concentration of protein g / ml = 1.55 × A 280 -0.77 × A260, Where A = absorbency

Determination of bacteriocin activity at different conditions

The effect of pH[20], temperature[21], proteinase K and lysozyme[22], and EDTA[23]on purified bacteriocin activity against bacteria were estimated.

Determination of bacteriocin molecular weight

This was estimated by electrolysis by using a poly-acrylamide gel[24,25].

Antimicrobial activity of bacteriocin

The antibiotic susceptibility testing was done by the well diffusion method[26]. The wells were prepared using sterile yellow tips and filled with 100 µl of extracted bacteriocin. The plates were placed in an incubator for 18hours at 37 ºC.

Results

Identification of studied bacteria

All bacterialisolates identified by 16S rRNA showed the similarity of 99% when it blasts in the NCBI database. 15 isolates showed mutation change at different loci were registered at GenBank of NCBI and given the following accession numbers (Table 1).

Screening the bacteriocinogenic isolates

Out of 63 *B. cereus* used in this study, 26 (41.26%) strains showed bacteriocin production activity. Screening of bacteriocine was done against four bacterial species. Some of the isolates showed maximum inhibition activity.

Table 1. Strains of *Bacillus cereus* that registered at GenBank and their accession numbers.

No. Of sample	Nucleotide change	Accession number	No. Of sample	Nucleotide	Accession number
BBS1	T>G, G>C	MK468691	BBS8	T>G,G>C,T>C	MK468736
BBS2	G>A,T>G	MK468692	BBS9	T>G	MK468798
BBS3	G>C,G>C	MK468693	BBS10	T>G	MK471340
BBS4	G>A	MK468700	BBS11	G>A	MK468901
BBS5	T>G	MK468704	BBS12	T>G	MK468902
BBS6	T>G,A>C	MK468727	BBS	G>C,G>C,G>C,G>T	MK480518
BBS7	C>T,G>A,T>C,G>C	MK468732	BBS13	C>T ,A>T, A>C, C>T, A>C, C>T, C>G, C>T, C>G, C>T	MK949281

Molecular detection of bacteriocin Cerecidin (*cer A)* gene

The bacteriocin cericidine A gene was found in 2 isolates (7.69%) out of 26 isolates as indicated in figure (1).

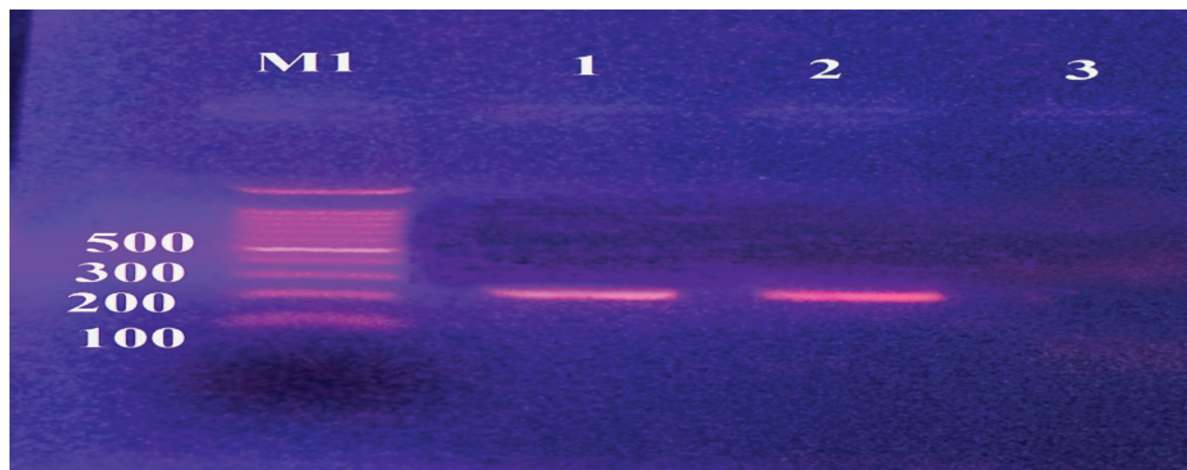

Figure 1. Detection of bacteriocin *cerA* gene by PCR .Lane M1= molecular marker; Lane 1-2=Positive for Cerecidin*cer A* gene approximately 233 bp; Lane 3= negative for Cerecidin*cer A* gene

Biological activity of crude bacteriocin

The crude bacteriocin was produced by *Bacillus cereus*cultured grown in BHIB for 18-24 hrs. Using ammonium sulfate and dialysis.The biological activity of crude bacteriocin was tested against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* spp. The results showed activity against *Bacillus cereus* and *Staphylococcus aureus* but no activity was detected against *Escherichia coli* and *Salmonella* spp. Table (2).

Table 2. Antimicrobial activity of crude bacteriocin against different types of bacteria

Types of bacteria	Inhibition zone (mm)
Bacillus cereus	10 mm
Staphylococcus aureus	6 mm
Salmonella spp.	0 mm
E. coli.	0 mm

Estimation of extracted bacteriocin concentration

The results show that the concentrations were ranged between 217.308 to 648.606 (Table 3).

Table 3. Estimation of bacteriocin concentration

No. of samples	260 nm	280 nm	Bacteriocin conc. gm/ml
Crude	1	0.962	217.308
1	2.570	2.680	648.606
2	1.986	2.290	596.2628
3	2.860	2.999	555.828
4	1.972	2.060	461.0536

Physical and chemical properties of bacteriocin

Thebacteriocin was active in a range from pH 3-11, with a best antimicrobial activity at pH 7 when tested on Gram-positive bacteria, *B. cereus* and *staph aureus*.

The bacteriocin was active at 30 °C on *B. cereus*,and *S. aureus* and lost its activity at high-temperature Table (4).

Table 4. The effect of different pH on bacteriocin

Isolates	pH			Temperature			
	pH 3	pH7	pH 11	30 °C	50 °C	70 °C	90° C
B. cereus	6 mm	11 mm	5 mm	+	-	-	-
S. aureus	6 mm	8 mm	5 mm	+	+	+	-

The bacteriocin lost its activity on *B. cereus* and *S. aureus* when treated with proteinase K and lysozyme.The addition of EDTA increases the activity of bacteriocin. The results showed high activity against *B. cereus* and *S. aureus* and Gram-negative *E. coli* and *Salmonella* spp. Table.

Estimation of the molecular weight of bacteriocin

The molecular weight of the protein was estimated by electrolysis by using a poly-acrylamide gel according to Lammeli method**.** The estimated molecular weight of bacteriocin was 47-54 KDa.

Discussion

*Bacillus cereus*produced a peptide that showed antimicrobial activity against major food-borne bacteria[27].Our results suggest that this substance has a bactericidal effect against *B. cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* spp. by inhibition the growth of these bacteria. The effect may depend particular test conditions, such as the quantity and purity of the bacteriocin, the indicator strain and concentration of cells[28].

In this study, the bacteriocin was purifiedand the activity of this crude bacteriocin appears against Gram-positive bacteria 10 mm and 6 mm for *B. cereus* and *S. aureus,*respectively. There was no activity on Gram-negative bacteria. In this study, thebacteriocin was active in a different pH value, but the maximum activity was found at pH 7 when tested on Gram-positive bacteria, *B. cereus* and *staph aureus*. The stability of bacteriocins at various pH scales is a limiting factor for their use in food[29].In this study, the activity of bacteriocin was lost when the temperature was increased. This was agreed with Sankar*et. al.,*[29].

In vitro, the present study shows that EDTA with bacteriocin used against Gram-negative bacteria to boost antimicrobial activity[30]. The gram-negative bacteria are poorly sensitive to bacteriocin and require increased concentration to inhibit growth. The bacteriocin was treated with EDTA to increase activity against Gram negative bacteria. The combination of bacteriocin and EDTA showed better antimicrobial activity. The bacteriocin lost its activity on *B. cereus* and *S. aureus* when treated with proteinase K and lysozyme, no inhibition zones are formed[30].In this study, single band of the purified bacteriocin appeared in SDS-PAGE. The product has a molecular weight ranging from 47-53 kDa, whereas in another study, the bacteriocin determined by SDS PAGE is 23 kDa[22]. The bacteriocin produced by *Bacillus cereus* GN105 showed a bacteriocin band at 3.5 kDa[31], at21 kDa[32]. and 43 kDa[12].

Conclusion

Several studies were done on *Bacillus cereus*in the similar area of study[33,34,35,36,37]. Bacteriocin production rarely investigated which considered as effective food preservatives. Though cerecidine A is the only the detected bacteriocin in this study. Bacteriocins have inhibitory action against food-borne pathogens such as *Bacillus cereus* and *Staphylococcus aureus*. The action of bacteriocinincreased by combining with EDTA.

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

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

Funding: Self-funding

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The Diagnostic Efficacy of Visually Enhanced Lesion Scope (Velscope) In Identifying Benign , Dysplastic and Cancerous Oral Lesions

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Abstract

Background : Visual inspection by conventional oral examination (COE) has been the backbone of oral cancer and precancer detection. More recently, several commercially available diagnostic adjuncts have been developed to aid clinicians in the early detection of oral epithelial dysplasia (OED) and squamous cell carcinoma (SCC), such as OralCDx, Toluidine coloring, ViziLite machine, Identafi machine, and VELscope.

VELscope is a technology based on the principles of autofluorescence mechanism imaging. This device offers in-vivo, real-time, direct visualization of tissue autofluorescence, termed direct visual fluorescent examination (DVFE). It is currently marketed as an oral cancer screening tool to be used with all new and recall dental patients and as an aid for surgeons in tumor margin delineation.

Aim of the study :was to obtain auto fluorescent data on variety of histopathologically distinct oral lesions and assessment of direct auto fluorescent examination validity in identifying dysplastic (premalignant) and malignant oral mucosal lesions using VELscope and judgment with histopathological examination.

Materials and methods: Routine oral examination of fifty patients having suspicious oral lesions followed by direct autofluorescent examination by VElscope and then histopathological examination.

Conclusion : VELscope can be considered as an adjunctive device to enhance the visualization of oral mucosal abnormalities, but not as a tool for risk stratification.

***Key words**: Autoflouresence examination , VELscope , oral premalignant lesions.*

Introduction

Several studies were conducted in many countries intrested with the diagnostic value of visually enhanced lesion scope (VELscope) since its development by the British Columbia Cancer Agency and granted FDA clearance on July 06, 2006 [1].Howerver no study was conducted in Iraq,so the current study may be considered the first study concerning with this field.

Biopsy and microscopic evaluation by a trained oral and maxillofacial or head and neck pathologist, remain the clinical gold standard to determine the presence and grade of dysplasia or carcinoma,but over time various diagnostic adjunct were developed[2].Autofluorescence imaging occupies the intrest of many researechers and spacialistsinthefieldofearlydetectionoforalpremalignant and malignant lesions,so several autofluorescence devices have become commercially available since the past decade till nowadays,such as VELscope (LED

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The principle of Velscope work depends on the changes of autoflouresent characteristic of the oral mucosa that contains abundant endogenous autofluorescence molecules called Fluorophores which are molecules that emit energy in the form of fluorescence when excited by light, including structural proteins such as collagen and elastin, the metabolic co-factors nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide(FAD),as well as several aromatic amino acids, and porphyrins .This should be done through blue excitation light (wavelength of 400–460 nm)emitted from the VELscope contacts these fluorophores,The level of fluorescence energy emitted from the endogenous autofluorescence substances will become lower than that of the light energy emitted from the VELscope, and green light with a wavelength of 515 nm is then emitted from endogenous autofluorescence substances in healthy area[5].

Dysplasia and cancer causing measureable changes in tissue autofluorescence in which the collagen cross-links and basal lamina of tissue affected by SCC and epithelial dysplasia are destroyed, and glucose is highly consumed in malignant tissue even when in an aerobic environment (Warburg effect). Also, certain changes, such as increased metabolism, nuclear pleomorphism, increased epithelial thickness, breakdown of collagen cross-links, increased vascularization and production of fluorophores by bacteria may also contribute to this effect[6].

Epithelial fluorophores concentration decrease in the epithelial dysplasia therefore, the lower level of endogenous auto fluorescence substances in tissue affected by epithelial dysplasia and SCC of the oral mucosa, which has no fluorescence energy, resulting in the appearance of a black area detected as fluorescence visualization loss (FVL)[7]. So in order to perform simple criteria analysis and evaluation of the tissue status, the sample interpreted correctly by Velscope and further analyzed by histopatholgical examination .

The advantages of auto fluorescence examination using VELscope include obtaining of diagnostic information in situ, real time, and in a minimally invasive manner [8]. High sensitivity for dysplasia and cancer, ability for assessment of large areas of oral mucosa, consumables are not required, clinical utility for risk assessment during longitudinal monitoring of patients with known high-risk potentially premalignant oral lesions or previous history of cancer and commercially available device rely on subjective interpretation of autofluorescence[6].

But, Velcsope is unfortunately limited by false positive results (low specifity) because lesions of various etiologies have different autofluorescent properties [9].

Subjects, Materialsand Methods

This cross sectional study was conducted fromthe 1st of February till the 1st of August 2019 in Oral Medicine Department at the College of Dentistry University of Baghdad.

Ethical approval and official permission was obtained from college of dentistry /university of Baghdad Scientific Committee.

Participation consent form for each subject was signed after brief details about the study, having the wright of withdrawing from the study at any time.

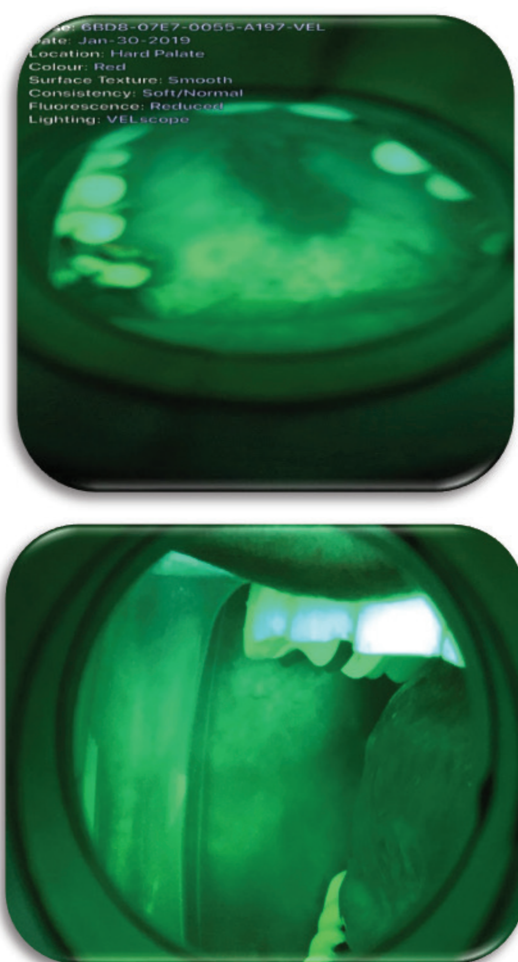
The current study involves 50 patients with an age ranged between 18-75 years with suspicious oral lesions. A case sheets were used to record the personal information including age, sex, occupation, address, phone number and age of onset and duration of the oral lesions. Medical history, family history and history of smoking and alcohol consumption were also recorded.

Excluded patients were those who have contraindications for biopsy sampling, such as bleeding disorders or uncontrolled systemic diseases, diabetic patient, pregnant woman, patients with a confirmed diagnosis of dysplasia or malignancy in a previous biopsy, or patient with history of photosensitivity or those using photosensitive medication who should not exposed to the light emitted from the VELscope device were also excluded from the study.

All patients were examined by oral medicine specialist at oral diagnosis department for exploring the existence of the oral lesions which was done in sequence examination procedure of oral soft tissue following

directions represented by WHO (1987).

Direct visual examination is then performed by VELscope Lesional and perilesional tissue was assessed for visual fluorescence retention (VFR) and visual fluorescence loss (VFL) when viewed through the velscopeVX handpiece, As documented by the manufacture information ,(LED Dental Inc ,Vancouver ,Canada ,2007).figure (1)



Figurer (1) A:VELscope examination of hard palate demonstrating an area of VFL B: VELscope examination of buccal mucosa demonstrating an area of VFR

Direct visual examination findings were documented and digital photographs of tissue fluorescence were acquired. Images were obtained directly through the VELscope viewing handpiece using Ipod 5 digital camera connected to VElscope by image adapter that is based on the manufacturer (LED DENTAl Inc).

Biopsies were then taken from the suspicious area of the oral lesions,Specimens were stored in 10% neutral buffered formalin and sent to at oral pathology laboratory of the oral diagnosis department at the collage of dentistry /university of Baghdad and Al Byan private specialized medical laboratory.

To determine the sensitivity and specificity of the VELscope, attempts were made to have the biopsy site cover both the COE and VEL positive regions.

Following the biopsy, the tissue sample was fixed in 10% formalin and sent to The Oral Pathology Laboratory for processing and histologic diagnosis, without information of the DVFE findings.

Formalin-fixed and paraffin embedded tissue was processed and stained following standard protocol for routine (hematoxylin and eosin) histopathologic evaluation. The examination were done under a light microscope by an experienced specialist in oral and maxillofacial pathology ,and epithelial dysplasia classified according to its severity (mild ,moderate ,sever).

Final histopathologic diagnoses were recorded on data collection sheets.

To calculate the sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV), the results of both the COE and VELscope were compared with the histopathological results.Statistical analysis evaluation was performed using SPSS software.

Results

Results shows that female patients were formed 27(54.0%) with no significant different compared with male numbers 23(46.0%)at $P>0.05$.Age groups of the examined patients seems to be having an extreme shape distribution of type I (smallest values of left skewness),with mean and standard deviation values 53.90 yrs,and 10.44 yrs., as well as recorded highly significant different at $P<0.01$.

Table (1) shows distribution of risk factors in the studied group, such that: (Smoking status and Alcoholic)

Table(1):Risk Factors distribution with comparison's significant

Risk Factors	Groups	Patients		C.S.
		No.	%	
Smoking	No	36	72	P=0.003 HS
	Yes	14	28	
	Total	50	100	
Alcoholic	No	45	90	P=0.000 HS
	Yes	5	10	
	Total	50	100	

The clinical appearance of the oral lesions on the conventional oral examination demonstrated in table (2)

Table(2): Appearance of oral mucosal lesions with comparisons significant

Appearance	Resp.	No.	%	C.S. (*)
White Lesion	No	25	50	P=1.000 NS
	Yes	25	50	
Red Lesion	No	38	76	P=0.000 HS
	Yes	12	24	
Ulcer	No	43	86	P=0.000 HS
	Yes	7	14	
Exophytic Lesion	No	43	86	P=0.000 HS
	Yes	7	14	
Swelling	No	47	94	P=0.000 HS
	Yes	3	6	

Result shows that most of the oral lesions presented as a white Lesion, and they are accounted 25(50.0%),with no significant different at P>0.05,then followed by "Red Lesion ", and they are accounted 12(24.0%),with significant different at P<0.01, then followed by "ulcer, and exophytic Lesion" and they are accounted for each 7(14.0%),with significant different at P<0.01, and finally only 3(6.0%) cases are recorded "Swelling" appearance, with significant different at P<0.01 Autoflourescen examination by VELscope was performed on all the 50 patients .

After histopathological examination result shows most patient's diagnosed were having benign oral lesions , and they are accounted 37(74 %),table(3)

Table (3) histopathological diagnosis of benign lesions

Definite Diagnosis	No.	%	C.S. (*)
Lichen Planus	32	64	P=0.066 NS
	17	36	
Hyperkeratosis	44	88	P=0.000 HS
	6	12	
Chronic Inflammation	47	94	P=0.034 HS
	3	6	
Nonspecific ulceration	47	94	P=0.000 HS
	3	6	
Fibrolipoma	49	98	P=0.000 HS
	1	2	
Hyperplasic Epithelium	47	94	P=0.000 HS
	3	6	
Fibroma	49	98	P=0.000 HS
	1	2	
Papilloma	48	96	P=0.000 HS
	2	4	
Mucocele	49	98	P=0.000 HS
	1	2	

dysplastic lesion accounted 7 (14 %) and classified as mild dysplasia 5 cases ,moderate dysplasia 1 case ,sever dysplasia 1 case and SCC accounted 6 (12%) cases ,table 6 demonstrated the distribution of histopatholgical diagnosis of dysplasia and SCC.

Table(4)Histopathological distribution of dysplastic and SCC lesions

Diagnosis	Resp.	No.	%	C.S. (*)
Mild Dysplasia	Absent	45	90	P=0.000 HS
	Present	5	10	
Moderate Dysplasia	Absent	49	98	P=0.000 HS
	Present	1	2	
Sever Dysplasia	Absent	49	98	P=0.000 HS
	Present	1	2	
SCC	Absent	44	88	P=0.000 HS
	Present	6	12	

Autoflourescence examination of 37(74%) benign lesions by VELscope show that 24(65%) show VFR to be reported as true negative cases ,and 13(35%) lesions show VFL which is a false positive cases .

Dysplastic lesions examination show that 6(86%) lesion exhibit VFL which are true positive cases and 1(14%) exhibit VFR which is false negative case, sequmous cell carcinoma show 4(67%) true positive cases that axhibit VFL and 2(33%) false negative cases that exhibit VFR.As a result the autoflourescene examination by VELscope in this study show a sensitivity and specificity of 76.92% and 64.86% respectively,positive predictive value(PPV) is 43.48 %,And negative predictive value (NPV)88.88 %.Table(5)

Table (5): VElscope Sensitivity,specificity,PPV and NPP

Histopathological diagnosis	VFR-	VFL+	Sens	spec	PPV	NPV
Benign	24	13	76.92 %	64.86%	43.48	88.88
Dysplasia	1	6				
SCC	2	4				

Discussion

Oral cancer continues to be a major disease with significant morbidity and mortality so prevention and early detection are essential to improving health outcomes,thus,various diagnostic adjunct had been developed to improve the early detection capabilities of the potential oral premalignant and malignant lesions .among these adjunct is VElscope (visually enhanced lesion scope) that occupies a remarkable concern among researchers and specialist which is based on tissue autofluoresence characteristics.

To the best of our knowledge the current study considered to be the first one that's conducting in Iraq in an attempted to expand some of the early investigative prospect in the field of oral diagnosis .

The previous studies in this field reported sensitivities and specificities for the device in specialist practice range from 30% to 100% and 15% to 81% respectively [10]

Amirchaghmaghi et al,(2018)demonstrated a high sensitivity(90%)and low specificity (15%)in their study[11]

Koch et al.,(2017) reported a higher sensitivity(97%) and specificity of (95.8%) of the VELscope in diagnosing OSCC.[12] On the other hand,Rana et al.(2012) in their study compared VELscope examination with COE and reported that using the VELscope leads to higher sensitivity (100% vs. 17%), but a lower specificity (74% vs. 97%).[13]

The statistical analysis in the current study revealed the sensitivity value of the VELscope examination to be(76.9 %) that was warranted to the false negative results presentation,because one of 7 epithelial dysplasia cases and 2 of 6 SCC cases showed visual fluorescent retention which considered as false negative finding.

This result was also expressed by McNamara et al.,(2018) they found that a number of benign lesions displayed VFL[14].

The involvement of few cases of OPMDs and OSCC in the current study may also contribute to these results .

This is in agreement with results reported by Paderni et al. study,they showed a sensitivity of 75% [15], and disagreement with a study conducted by Farah

et al 2013 .[16], and Mehrotra et al 2010.[17] whom reported low sensitivity for this device(30% and 50% respectively) .

Specificity of VELscope examination that reported by the present study was 64.86%.The study demostrated that 13 of 37 benign lesions show VFL that's considered as false positive results. One of the main etiologies may be the inclusion of inflammatory and ulcerative lesions in the current study.This low specificity reflects VELscope weakness in distinguishing high-risk lesions(i.e., lesions with dysplasia and malignancy)from low-risk lesions(i.e., inflammatory and benign lesions without dysplasia) that's because of presentation of loss in fluorescent characteristic during the examination (black to grey color comparing with the adjacent tissues) with some cases of atrophic and erosive lichen planus ,ulcerative lesion, and hyperplastic epithelium .

In inflammatory and erythematous tissues, the destruction of structural molecules is less common; however, two factors contribute in the above mentioned results; an increased hemoglobin concentration due to increased circulation and an increased density of chronic inflammatory cells, such as lymphocytes, that's lead to increased dispersion and absorption of the fluorescence along with a darkening of the lesions.[18]

Conclusion

Direct visual fluorescent examination by VELsope has potential as a simple, cost-effective screening, biopsy guidance that act as an intermediate adjunctive tool between routine oral examination and a biopsy for oral premalignant and early malignant lesions and to be especially useful to alleviate both patient and practitioner concerns regarding a clinically oral suspicious lesion.

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

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

Funding: Self-funding

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Registered with Registrar of Newspapers for India
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Published, Printed and Owned : Dr. R.K. Sharma
Printed : Printpack Electrostat G-2, Eros Apartment, 56, Nehru Place, New Delhi-110019
Published at: Institute of Medico Legal Publications Pvt. Ltd., Logix Office Tower, Unit No. 1704, Logix City Centre Mall Sector- 32, Noida - 201 301 (Uttar Pradesh) Editor : Dr. R.K. Sharma, Mobile: + 91 9971888542, Ph. No: +91 120 429 4015

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