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CONTENTS

PREVALENCE AND COMPARISON BETWEEN THE EFFICACY OF DIFFERENT TECHNIQUES IN DIAGNOSIS OF *TRICHOMONAS VAGINALIS* IN ERBIL-IRAQ

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NARMIN RAFIK**

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ABSTRACT

Background and objectives: This study investigated the prevalence of *Trichomonas vaginalis* among females attending the gynaecological out patients clinics in Maternity hospital, several health centers and some private clinics in Erbil-Iraq, from 16th July 2007 till 15th July 2008, by using different diagnostic methods. In addition, a comparative study to evaluate the efficacy of different tests for diagnosis of infection.
Methods: Different diagnostic laboratory tests were used in the study like direct vaginal and urine examination, cultivation in Diamond modified broth, Papanicolaou (Pap) smears and measurement of vaginal pH value
Results:*Trichomonas vaginalis* detected only among 5 out of 1296 examined vaginal swabs, the rate of infection was 0.39% (0.42% in urban and 0.28% in rural regions). The highest rate of infection was among women other than single and married groups (i.e. widow, divorced and separated women), and among women of child-bearing age 14-40 years, with college education. The employers revealed higher seropositivity than the housewives. Trichomoniasis was higher among the pregnant than non pregnant women. The seropositivity rate was 100% among females with high vaginal pH levels 6, 6.3 and 6.6.
Pap smears revealed the highest infection rate 6.52%. Comparison between efficacies of different methods was determined and direct vaginal examination showed higher rate of positivity than cultivation technique, and Pap smear was more efficient in comparison with direct examination and cultivation of vaginal discharges, while no positive cases were detected by urine examination.
Conclusions:In comparison between serological methods and direct vaginal examination, complement fixation and ELISA methods were more efficient and the statistical difference was highly significant. The rate of seropositivity among the housewives was higher than the employers using the four laboratory methods.

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Key words: prevalence, comparison, diagnosis, *Trichomonas vaginalis*,Erbil.

Human trichomoniasis is a widely prevalent sexually transmitted disease of worldwide importance.

An estimated 200 million women suffer from trichomoniasis every year worldwide [1]. Maternal *Trichomonas* infection may also result in vaginitis, urinary tract infections, or respiratory distress in the premature newborn [2]. Epidemiologically, *T. vaginalis* infections are commonly associated with other sexually transmitted diseases (STDs) and may be a particularly sensitive marker of high risk sexual behavior.

Trichomoniasis is frequently seen concomitantly with other STDs, particularly gonorrhea [3].

Diagnosis can be made by different methods like pelvic examination, microscopic demonstration of trophozoites of *T. vaginalis* in wet mount of the sedimented urine and vaginal secretions of female and males, it may be found in urine and prostatic secretions, using acridine-orange, Papanicolaou and direct fluorescent antibody (DFA) staining methods, vaginal pH testing, culture tests, enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction PCR [4].

The aims of the study were to determine the prevalence of trichomoniasis in women in Erbil governorate and to compare between the efficacy of different direct examination and serological tests for diagnosis of *Trichomonas* seropositivity.

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METHODS

Time and location

One thousand two hundred and ninety six female patients were enrolled in the study, between 16th July 2007 and 15th July 2008, for detection of *T. vaginalis* infection, they were attended to some private clinics, gynecological out patient's clinics in Maternity hospital and several health centers in Erbil Governorate.

Collection Of Specimens

Genital tract examination was carried out for each patient, two high vaginal swabs, for each patient, were obtained by a sterile cotton swab; one was kept in 3 ml saline solution for direct microscopic examination, the other swab used for culture (Diamond Modified Broth). For unmarried females low vaginal swabs were taken. Then each tube was labeled with the patients name or number and date of collection.

Estimation of vaginal discharge pH was done by pH-indicator paper (Whatman International Ltd Maidstone, Germany) on which the discharge was placed for few seconds and the resultant color was compared with the color segments printed on the strip. Each patient was asked to collect urine in a clean container for general urine examination to determine the urinary tract involvement also.

Papanicolaou (Pap) smears were obtained from ninety two patients, fixed, stained in Harris's haematoxylin and eosin stains, dehydrated, mounted and examined for detection of *T. vaginalis* [5].

Cervical smears were taken from ninety two females by using sterilized bivalve speculum which introduced into the vagina, then the small end of the cervical wooden scraper (Ayre spatula) was placed in the external os of the endocervical canal as deeply as possible and rotating it 360 degrees, energetically scraping the entire surface of the external os and part of the internal os. The ectocervical smear was taken by using the opposite wider end of the scraper [1].

Cultivation

The medium used for cultivation of *T. vaginalis* was Diamond Modified (Que-Bact Laboratories-Technical Data 2491/Canada).

Detection of anti-*Trichomonas vaginalis* antibodies was done using modified ELISA (BioCheck Inc. CA).

Complement fixation test was done according to Santa Cruz Biotechnology, Lot No.: 1207, Germany.

Statistical analysis

The statistical analysis was conducted using the software program Statistical Program Social System (SPSS version 13.0).

The comparison was conducted between positive and negative values for all parameters using Chi-square test as mentioned by Milton and Tsokos [7].

RESULTS

Out of 1296 examined vaginal swabs, 942 females were from urban and 354 from rural areas, only 5 cases were positive for T. vaginalis (4 in urban and 1 in rural areas), the total rate of infection was; 0.42% in urban and 0.28% in rural areas (Table 1).

Table 1. Prevalence of *T. vaginalis* according to the residency of the patients.

Residency	No. examined	No. +ve	%
Urban	942	4	0.42
Rural	354	1	0.28
Total	1296	5	0.39

Regarding the marital status, the rate of infection was 13.33% among 15 patients other than single and married one (i.e. widow, divorced and separated women), 8.33% among 12 single (unmarried) females and 0.16% between 1269 married women as shown in (Table 2).

Table 2. Prevalence of *T. vaginalis* according to the marital status of the patients.

Marital status	No. examined	No. +ve	%
Single	12	1	8.33
Married	1269	2	0.16
Others	15	2	13.33
Total	1296	5	0.39

Others=Widow, Divorced and Separated Women.

Regarding the patient's age, (Table 3) shows that the patients mostly infected by *T. vaginalis* were at their child-bearing age 14-40 years.

Table 3. Prevalence of *T. vaginalis* according to the patient's age.

Age (years)	No. examined	No. +ve	%
14-24	384	2	0.52
25-35	597	2	0.34
36-46	270	1	0.37
47-57	41	0	0
58-68	4	0	0
Total	1296	5	0.39

(Table 4) shows prevalence of *T. vaginalis* according to the educational status of the patients and the lower infection rates of trichomoniasis were almost evenly distributed among women who had school education and illiterate patients (0.36% and 0.35% respectively) as compared to 0.57% with college education.

Table 4. Prevalence of *T. vaginalis* according to the educational status of the patients.

Education level	No. examined	No. +ve	%
Illiterate	283	1	0.35
School	839	3	0.36
University	174	1	0.57
Total	1296	5	0.39

(Table 5) illustrates that the employer women were more susceptible to trichomoniasis than housewives (0.53% and 0.36% respectively).

Table 5. Prevalence of *T. vaginalis* according to occupation of the patients.

Occupation	No. examined	No. +ve	%
Housewife	1106	4	0.36
Employee	190	1	0.53
Total	1296	5	0.39

(Table 6) Indicate that *T. vaginalis* infection was more prevalent in pregnant women as shown the higher infection rate (0.41%) as compared to non-pregnant women (0.10%).

Table 6 . Prevalence of *T. vaginalis* with regard to gestation.

Type of gestation	No. examined	No. +ve	%
Pregnant	246	1	0.41
Non-pregnant	1023	1	0.10
Total	1269	2	0.16

Different laboratory tests were used in this study, to demonstrate the prevalence of *T. vaginalis* among female's patients (Table 7). Out of 1296 females, examined by direct vaginal swab, only 5 of them were infected (0.39%). While 3 cases (0.25%) were positive for *T. vaginalis* by examination of 1210 urine samples. The vaginal discharges of 223 women were cultivated in Diamond modified broth and 3 positive cases (1.35%) were recorded, while from 92 Papanicolaou smears, 6 women (6.52%) gave positive results for *T. vaginalis* excluding 3 females, who were infected by *T. vaginalis* using direct vaginal examination, because one female was unmarried, the other was pregnant, and the third woman refused to participate in the Pap study. The pH of vaginal discharges of 1061 patients was measured and only five females of high vaginal pH were infected with *T. vaginalis* (0.47%).

Table 7. Prevalence of *T. vaginalis* among females patients with vaginal discharges using different laboratory tests.

Test	No. examined	No. +ve	%
Direct vaginal examination	1296	5	0.39
Urine examination	1210	3	0.25
Culture	223	3	1.35
Pap smear	92	6	6.52
Vaginal pH	1061	5	0.47

(Table 8), shows that the infected women with *T. vaginalis* were of high vaginal pH values 6, 6.3 and 6.6 (100% for each).
In vitro experiments designed to determine the effect of different pH values on trichomonads survival and metabolism

Table 8: Prevalence of *T. vaginalis* according to the vaginal pH of the patients.

pH	No. examined	No. +ve	%
3.8	6	0	0
4.2	5	0	0
4.4	38	0	0
4.6	256	0	0
4.9	411	0	0
5.2	195	0	0
5.5	145	0	0
6.0	2	2	100
6.3	2	2	100
6.6	1	1	100
Total	1061	5	0.47

Regarding comparison of the diagnostic methods used in this study, the efficacy of direct vaginal examination and cultivation method was performed on 223 patients; rates of infection were 1.79% and 1.35% respectively (Table 9).

Table 9.Comparison between efficacy of direct vaginal examination and culture in detection of *T. vaginalis.*

Test	No. examined	No. +ve	%
Direct vaginal examination	223	4	1.79
Culture	223	3	1.35

Comparison between efficacy of direct vaginal examination, urine examination, culture and Pap smear among 61 patients was illustrated in (Table 10) and higher infection rate was (4.92%0 for Pap smears.

Table 10. Comparison between efficacies of direct vaginal examination, urine examination, culture and Pap smear among 61 patients.

Test	No. +ve	%
Direct vaginal examination	2	3.28
Urine examination	0	0
Culture	2	3.28
Pap smear	3	4.92

Comparison between efficacy of direct vaginal examination, ELISA, and complement fixation tests in detection of trichomoniasis is shown in (Table 11). There were highly significant differences in the efficacy of the three diagnostic methods used; complement fixation and ELISA tests showed higher infection rates 60% and 52% respectively than direct vaginal examination 0.39% which has low sensitivity, varies from 38% to 82.

Table 11. Comparison between efficacies of direct vaginal examination, ELISA, and complement fixation tests in detection of *T. vaginalis* infection.

Test	No. examined	No. +ve	%
Direct vaginal examination	1296	5	0.39
ELISA	25	13	52
Complement fixation test	15	9	60
$\chi 2=587.523*$	df=2	P<0.01	

** Significant*

DISCUSSION

The infection rate, in the present study 0.39% is much lower than those reported by some other studies in different parts of Iraq, but they still represent an important level from the public health point of view. In Baghdad, the infection rates of trichomoniasis was 9.46% [8] ; in Mosul [9]

33%; and 13% in Basrah. In Tikrit and Kirkuk several studies were carried out by [11-13], they recorded infection rates of 4.3%, 1.33% and 78% respectively. In another study in Kirkuk a lower rate 2.8% was reported [14], while in Sulaimani, a much lower rate 1.6% was reported [15].

The frequency of *T. vaginalis* has diminished markedly in the present study, raising the hypothesis that this may be a consequence of the improvement in conditions of hygiene and sanitary habits.

This study showed a lower infection rate of trichomoniasis among women in the rural areas 0.28% as compared with that of urban region 0.42%. This is in agreement with the study of Al-Somaeday [12] in Tikrit who reported rates for rural and urban (0% and 2.06%) inhabitants, respectively. While disagreed with the results of Al-Zubaidi [16] in Mosul, she indicated higher infection rate among women living in the rural areas.

In general, socioeconomic factors such as low level of education or economic income, and strong sexual desire are associated with a higher prevalence rate of trichomoniasis [17]. However, in the present study, it was found that a lower level of education or economic income tended to confer protection against trichomoniasis, in the rural area.

The rate of infection was 13.33% among 15 (widow, divorced and separated women), 8.33% among 12 single females and 0.16% among married women These results disagree with those of Kadir [11] in Tikrit and Fattah [15] in Sulaimani, who recorded the highest rate of infection among married women 3.74. The high infection rates in widows and unmarried females may be due to the small sample size taken from these patients.

Regarding the patient's age, the patients mostly infected by *T. vaginalis* were at their child-bearing age 14-40 years. This is in agreement with that reported by Al-Mudhaffar [18], from Baghdad, who showed that the highest rate of infection 10.7% among women with age group 20-40 years, and also with Kadir [11] in Tikrit city, and Mahdi et al. [10] in Basrah that the child-bearing bearing age 14-40 years. The presence of infection with *T. vaginalis* in women of reproductive age may be related to the high level of sexual activity which increases the level of reproductive hormones and decreases with progression of age [19]. In addition, there are many factors which cause differences in rates of infection among different age groups like the pH of vagina, secretion of estrogen and progesterone hormones for maintaining the pH of the vagina through the birthing age as well as abortion and frequency of pregnancy and immunodeficiency of the body defense after menstruation period [20].

The lower infection rates of trichomoniasis were almost evenly distributed among women who had school education and illiterate patients 0.36% and 0.35% respectively compared to 0.57% with college education. This finding is in agreement with Mahdi et al. [10] in Basra, who recorded the rates of *T. vaginalis* infection 10.6%, 12.2% and 18.6% among low, moderate and high education levels respectively

The employed women were more susceptible to trichomoniasis than housewives 0.53% and 0.36% respectively and this may be due to the fact that the employer women are more obligated to use contraceptives, thus they are sexually more active than the housewives. In a study performed by Darogha [21], she observed that *T. vaginalis* infection was more common among women who used contraceptives 60.2% than those who did not use any kind of contraceptives 39.8% in Erbil city. This finding is in agreement with the study of Mahdi et al. [10] in Basra, who detected the infection rate by *T. vaginalis* 18.4% in the workers outside home and 11% in the housewives.

The higher infection rate of in women during gestation and pregnancy 0.41% in comparison to non-pregnant women 0.10% is also reported by several researchers, such as Mahdi et al. [10] in Basra, 18.6%,

Mawlood [22] in Erbil 21.65%, Sulaimany governorate 1.6% and Bebany [13] in Kirkuk and Tikrit cities 48% and 22.22% respectively. While the results of the present study disagreed with the findings of other studies which recorded the lower infection rates with trichomoniasis among pregnant women such as Kadir et al. [11]; Darogha [21] 9.1% and 5.1% respectively.

The higher rate during pregnancy may be attributed to the hormonal disturbances during pregnancy period such as hyperestrogenism and an excess of glycogen levels in vaginal mucoid since it is a suitable environment for the growth and multiplication of the parasite .

The high vaginal pH values 6 of infected women are similar to values of pH 5-6 and 6 were recorded by Kanno and Sobel [24]. Sulyman [14] reported the infection rates 6.7%, 2.4% and 2.0% among infected women with vaginal secretion pH values of 7-8, 5-6 and 3-4 respectively in Kirkuk city.

In vitro experiments designed to determine the effect of different pH values on trichomonads survival and metabolism, Diamond [25] indicated that this parasite grow optimally at a pH of 6.0 – 6.3, and Connaris [26] observed that cultures buffered between pH 5 and pH 9 all had a pH of 6 after two day's growth and this result implies that this parasite has a mechanism for both raising and lowering the pH of the microenvironment. However, organism began to die below pH 5. The increase in vaginal pH causes a decrease or elimination of endogenous Lactobacillus species and thus creates a better environment for the growth of the parasite and *T. vaginalis* flourish best. In addition, there are certain other factors contributing to the pathogencity, such as cell-detaching factors, which are inactivated at a pH of less than 5 [27] .

Comparison between efficacy of direct vaginal examination and cultivation method; rates of infection were 1.79% and 1.35% respectively. The results disagree with several studies which demonstrated that the culture technique is more efficient than direct microscopic examination of vaginal secretions to diagnose trichomoniasis such as: Negm and el-Haleem [28] who reported 56.5% by wet mount microscopy and 72.9% by culture in Egypt. Sulyman [14] , in Kirkuk city, reported a rate of 2.4% by wet mount and 2.8% by cultivation method.

Comparison between efficacy of direct vaginal examination, urine examination, culture and Pap smear, (Table 10) shows higher infection rate 4.92% for Pap smears which is in agreement with Ryu et al. [29] , who observed similar result 4.6% in comparison with vaginal wet mount 2% and culture 3.3%; also in a group of 290 symptomatic patients with cervico-vaginitis and 160 asymptomatic women in Egypt, Mahmoud et al. [30] detected 35 positive samples using culture techniques, of these, 12 were positive by wet mount and 21 by Pap Smear.

In this study no positive cases (0%) were recorded by wet mount examination of urine. Several studies reported higher infection rate in vaginal swabs than urine [10, 11,20] .This is due to fact that genital tract is the normal habitat of *T. vaginalis.*

There were highly significant differences in the efficacy of the three diagnostic methods used; complement fixation and ELISA tests showed higher infection rates 60% and 52% respectively than direct vaginal examination 0.39% which has low sensitivity, varies from 38% to 82%, due to several reasons, it is dependent on the inoculum size because fewer than 104 organisms/ml will not be seen. This finding is in comparable with Kharofa [9] in Mosul, who demonstrated infection rates with trichomoniasis by using ELISA method 64%, and direct microscopic observation of motile protozoa from vaginal samples 33%.

It is concluded that trichomoniasis in women is not highly prevalent in Erbil Governorate. Higher infection rate was in urban inhabitants, women of reproductive or child-bearing age,

employer and with college education level. Single and married women showed lower infection rate than other women. High pH values of vaginal discharges were recorded among trichomonad infected women.

Pap smears revealed higher efficacy in comparison with direct vaginal examination and cultivation techniques, Serological methods were highly significant diagnostic methods in comparison with direct vaginal examination.

It is recommended to carry on further studies, on patients attending the primary health care centers and gynecological clinics in different parts ofr the country.

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پوختە

ڕێژا بەلاڤبوونێ و بەراوردکرنا بکێرهاتنا ڕێکێن جودا بێن دەستنیشانکرنا ترایکوموناس ڤاجاینالس ل هەولێرێ–عیراق

دڤێ ڤەکولینێ دا ڕێژا بەلاڤبوونا ترایکوموناس هاتە تاقیکرن دناڤ ئافرەتێن سەرەدانا کلینیکا دەرڤە یا نەخوشخانا زاروکبوونێ و هندەك بنگەهێن ساخلەمی و کلینیکێن تایبەت ل هەولێرێ هەر ژ 16ی تیرمەها 2007ێ هەتا 15ی تیرمەها 2008ێ بکارئینانا ڕێکێن جودا بێن دەستنیشانکرنێ، زێدەباری بەراوردکرنا بکێرهاتنا وان ڕێکان.

ئەڤ ئێشە هاتە دیتن لدەف 5 ژ 1296 نمونێن هاتینە تاقیکرن کو ڕێژا توشبوونێ دبیتە 0.39٪ (0.42٪ لبارژێران و 0.28٪ لدەوروبەران. پرانیا وان ئەو ئافرەت بوون بێن پشتی شویکرنێ ماینە بێ هەڤژین ئەوێن دژیێ 14–40 سالی دا. هەروەسا ڕێژە پتر بوو لدەف ئافرەتێن کاردکەن ژ ژنێن بەرمالان.

ڕێکێن جودا بێن دەستنیشانکرنێ هاتنە بکارئینان, ولدەمێ بەراوردکرنێ دیار بوو کو ڕێکا نموونێ پاپ ژهەمیان پتر دکاریت ڤێ ئێشێ دەستنیشانکەت، و بکێرهاتنا ڤێ ڕێکێ بو دەستنیشانکرنێ باشتر بوو ژڕێکێن دی وەکی تاقیکرنا نموونێ ئێکسەر ژئافا لەشێ ئافرەتێ.

الخلاصة

معدل انتشار مرض الترايكوموناس فاجاينالس ومقارنة فعالية الطرق المختلفة لتشخيصه في أربيل–العراق

شمل هذا البحث دراسة معدل الاصابة بمرض الترايكوموناس لدى النساء في العيادة الخارجية لمستشفى الولادة و بعض المراكز الصحية والعيادات الخاصة في أربيل للفترة من 16 تموز 2007 الى 15 تموز 2008 باستخدام طرق مختلفة للتشخيص مع مقارنة فعالية هذه الطرق للتشخيص.

وجد المرض لدى 5 من بين 1296 من النماذج المهبلية التي تم فحصها و كانت نسبة الاصابة 0.39٪ (0.42٪ في الحضر و 0.28٪ في الأرياف). وكان المعدل الأكثر للاصابة بين النساء الأرامل والمطلقات او المنفصلات أكثر من غيرهن، واللواتي في العمر الانجابي 14–40 سنة. كذلك النسبة لدى الموظفات كانت أكثر من ربات البيوت.

استخدمت طرق مختلفة للتشخيص و عند المقارنة كانت طريقة شريحة باب الأكثر كفاءة في التشخيص من غيرها من الطرق مثل الفحص المباشر مسحة المهبلية.

EVALUATION OF ANTI-PHOSPHOLIPID ANTIBODIES IN YOUNG PATIENTS WITH THROMBOEMBOLIC STROKE

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ABSTRACT

Background and objective: To evaluate the antiphospholipid antibodies in young patients with thromboembloic stroke and to define the types and effective isotypes of some important antiphospholipids which are lupus anticoagulant, anticardiolipin and antiphosphatidyle serine antibodies, also to find any concomitant effect of some non antiphospholipid parameters.

Methods: A case control study performed in Ibn-Sina and Al-Salam Teaching Hospitals. Seventy five young patients (age less than 45 years) with thromboembolic stroke diagnosed by magnetic resonance imaging and without any clear recognizable factors. Detection of antiphospholipid antibodies was done for lupus anticoagulant and assay of anticardiolipin and antiphosphatidyl serine antibodies by enzyme linked immunoassay. Anti-nuclear antibodies, C-reactive protein and D-Dimer reaction were tested by immunoserological methods. Fifty healthy, age and sex matched blood donors as healthy volunteers were studied as control group.

Results: The detected cases with positive antibodies formed(26.7%),and the mean age was 32±12 year and male to female ratio 2:3. Lupus anticoagulant was positive in 8% of cases. Moderately and highly positive anticardiollipin antibodies (IgG and IgM) level were significantly more seen in patients with stroke than the control with P(<0.001) for each type of antibodies. Moderately and highly positive antiphosphatidyl serine antibodies (IgG and IgM) levels were significantly more seen in patients with stroke than the control with P value <0.001 for each type of antibodies. Low positive level for both anticardiolipin and antiphosphatidyl serine antibodies were of no value in young patients with thromboembolic stroke. High CRP level was of significant in young patients with thromboembolic stroke and positive antiphospholipid (P<0.05) . Positive D-dimer reaction was seen in positive antiphospholipid patients P(<0.001).

Conclusion: Moderately and highly positive antiphospholipid antibodies were shown to play an important role in the development of thromboembolic stroke in young patients less than 45 years.

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Key words: Antiphospholipid , Antibodies,Stroke,Young.

Anti-phospholipid antibodies (APLA) are heterogenous group of circulating serum polyclonal, immunoglobulins IgG and IgM that bind negatively charged or neutral phospholipids component of cell membrane and may induce thrombotic disorders [1].

The anti-cardiolipin antibodies (aCl-Abs) and lupus anticoagulant (LA) are the most two important antibodies to be studied. [2,3] Other autoantibodies against negatively charged phospholipids rather than (aCl) and (LA) were also mentioned as phosphatidylserine (aPS) [4,5].

The (LA) can be detected by lupus anticoagulant sensitive activated partial throboplastin time (APTT) or diluted Russel, S. Viper Venom Time (DRVVT), while (aCL, Ab) can be tested by enzyme linked immunoassay (ELISA) method for B2-glycoprotein dependent antibodies including IgG and IgM [2,6]. Such antibodies occur with undue frequency in young patients with stroke and or transient ischaemic attack and are not associated with concurrent diagnosis of systemic lupus erythematosis in most cases. [7] and in

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the absence of the usual important risk factors for cerebrovascular disease [8].

The aim of this study was to evaluate the APLA in young patients with stroke and to define the types and effective isotypes of some important APLAs which are (LA) , (aCl) and (aPS), also to find any concomitant effect of some non-APLA parameters.

METHODS

After proval was obtained from the local ethics committee and informed consent obtained from each patients,and this study was carried out on 75 patients admitted to the Neurology Units in Ibn-Sina and Al-Salam Teaching Hospital in Mosul during a period of 2 years (January 2010-January 2012).

The criteria for patient selection were less than 45 years of age and have no history of risk factors for stroke including smoking, diabetes mellitus, hypertension, prolonged immobilization, sickle cell disease, cardiac arrhythmia, family history of stroke and there was no laboratory evidence of hyperlipidaemia.

Full investigations were done including complete blood count, activated partial thromboplastine time, prothrombine time, lipid profile, renal and liver function tests, echocardiography, Doppler ultrasound of both carotid arteries and magnetic resonance imaging (MRI). The diagnosis of thromboembolic stroke was clinically made by neurologist and confirmed by MRI.

The APLAs were tested by estimation of IgG and IgM for both (aCl) and (aPS) antibodies by (ELISA) as (GPL and MPL unit /ml) (pharmacia and Upjohn, Freiburg, Germany). These antibodies were considered as negative (<15MPL or GPL unit/ml) low positive (15-25MPL or GPL unit/ml) and highly positive (>60 MPL or GPL unit/ml).

Blood samples for (LA) were collected in tube containing 3.2 trisodium citrate, centrifuged at 2500 xg for 15 minute within 30 minute of collection then centrifuged at 4oC to obtain platelet poor plasma, clotting based test for (LA) was done using start 4 (from Stago Diagnostica France).

The ANA, CRP, and D-dimer were tested. All the laboratory kits and reagents used in this study were purchased from international suppliers and companies. CRP(from plasmatic…) titre of >12mg/L was considered of significant value.D_dimer reaction(qualitative and semiquantitative) (from ATLAS ,UK)

Fifty healthy, age and sex matched blood donors as healthy volunteers were taken as control.

The proportions were compared using the chi-square test with df 1 or df(r-1)and student t- test wherever indicated. The significant level was set at $P<0.05$.

RESULTS

The detected cases with positive APLAs were 20 out of 75(26.7%). The age range of positive cases was 20-44 years (mean ±SD, 32±12 year), 12 female (60%) and eight male (40%), with male to female ratio 2:3. The detected frequencies of different types of APLAs in patients with stroke and the controls are shown in (Table 1).

Moderately and highly positive aCl (IgG and IgM) were significantly seen in young patients with thromboembolic stroke with P value <0.001 in comparison to control.

Moderately and highly positive aPS (IgG and IgM) were significantly seen in patients with thromboembolic stroke with P value <0.001 in comparison to control.

Low positive aCL and aPS antibodies (IgG and IgM) in both patients and control were not significantly different.

Lupus anticoagulant was of significance in young patients with thromboembolic stroke, with P<0.001. (seen in 8% of positive cases for APLA).

History of previous stroke was seen in 2 patients, previous deep venous thrombosis

in one case and 2 female had history of repeated abortion.

Other important none APLA parameters in both patients and control were shown in (Table 2).

High CRP level was significantly noticed in young patient with thromboembolic stroke and positive APLA in comparison to those with negative APLA and control (P<0.05).

Table 1. The frequency of different APLA in thromboembolic stroke and control.

Group	Patients with positive APLA		Control		P-value
Test	No.	%	No.	%	
aCL IgG (GpL unit)					
<15	**4**	**20**	**46**	**92**	**<0.05**
15-25	**3**	**15**	**2**	**4**	**N.S****
26-60	**7**	**35**	**2**	**4**	**<0.001**
>60	**6**	**30**	**0**	**0**	**<0.001**
aCL IgM (MpL unit)					
<15	**3**	**15**	**42**	**84**	**<0.001**
15-25	**3**	**15**	**4**	**8**	**N.S**
26-60	**10**	**50**	**4**	**8**	**<0.001**
>60	**4**	**20**	**0**	**0**	**<0.001**
aPS IgG (GpL unit)					
<15	**0**	**0**	**45**	**90**	**<0.001**
15-25	**5**	**25**	**5**	**10**	**N.S**
26-60	**4**	**20**	**0**	**0**	**<0.001**
>60	**11**	**55**	**0**	**0**	**<0.001**
aPS IgM (MpL unit)					
<15	**2**	**10**	**45**	**90**	**<0.001**
15-25	**4**	**20**	**5**	**10**	**N.S**
26-60	**3**	**15**	**0**	**0**	**<0.001**
>60	**11**	**55**	**0**	**0**	**<0.001**
LA	**6**	**8**	**0**	**0**	**<0.001**

** Chi-square test*

*** N.S: Not Significant*

Table 2. Comparison of other important parameters in patients with thromboembolic stroke and control

Group	Patients (20) positive APLA		Patients (55) negative APLA		Control (50)		P-value*
Test	No.	%	No.	%	No.	%	
Positive ANA	**3**	**15**	**0**	**0**	**0**	**0**	**N.S**
CRP(>24mg/L)	**8**	**40**	**6**	**14**	**2**	**4**	**<0.05**
Positive D-dimer reaction	**20**	**100**	**38**	**69**	**0**	**0**	**<0.001**

**chi-square test*

*** N.S: Not Significant*

Positive D-dimer reaction was significant in patients with APLA (P<0.05). High D-dimer concentration more than (400mg/dl) was detected in highly & moderately positive aCL and aPS antibodies. The mean concentration by semiquantitation of D-dimer in low positive cases was (260±20mg/dL).

MRI finding in patients with positive APLA showed multiple infarcted area in 5(25%), localized small infracted area was seen in 15(75%), left sided brain involvement was observed in 8(40%).

DISCUSSION

The association of APLA with stroke is still controversial [9, 10, 11, 12] and there are several prospective studies showing association while others have noticed

otherwise[1,13]. The mean age for cases with positive APLA was 32±12 year, Danagariya (14) et al noticed on comparing the APLA positive and APLA negative stroke patients, that APLA positive patients had an average age of 36.8 years which was younger than average age for APLA negative group. The detected frequency of APLA in thromboembolic stroke in our study was (26.7%). Nagaraja et al in a study of 60 cases of young with stroke found elevated aCL in (23%). Other study have demonstrated the presence of APLA (LA, aCL or both) in (41%) with stroke. [16]

Lupus anticoagulant was detected in nearly 8% of patients and it was of significance.

Mishra and Rhohatg (2009) noticed that both LA and APLA showed significant correlation with the occurrence of stroke in young patients [16] which was different from the observation of Gat et al who conclused that measuring LA is helpful to define a patient's risk for arterial and venous thrombosis[17]. Lupus anticoagulant was detected in nearly 20% of patients by Mishra et al.

Moderately positive and highly positive aCL and aPS were significant in young patients with thromboembolic stroke. Ali and Abdulla [18] demonstrated an association between aCL(IgG and IgM) with concentration above 30 units/ml and a PS (IgG) and increased stroke and / or transient ischaemic attack risk. In other study elevation of aCL(IgG) was seen in 21.4% of patients and in 4% of control [16].

In other stroke study,they found ,patients with APLA, the risk of cerebral infarction was 2.3 times higher than in those negative for the antibody[19].

The highly positive concentration of APLA was detected in two cases with history of previous stroke. Van Goor et al noticed that antiphospholipid is not a strong risk factor for recurrent strokes, TIA or other thrombotic episode in young women with previous stroke in 28 consecutive patients with stroke or transient ischemic attack. Case-control studies of stroke in young people have been uniformly positive for APLA [20,21,22,23]; other case-control studies among older adults have found aPLs to be associated with ischemic stroke [24,25,26,27]. Robert J et mentioned that their study support the importance of antiphospholipid antibodies as an independent risk factor for stroke in young women.

The presence of positive anti-nuclear antibody in some of studied patients may by itself represent the existence of antiphospholipid syndrome and the frequency of this parameter differs between the stroke cases and control.

High CRP level more than 24mg/L among APLA positive cases was significant ($P<0.05$). Recently it has been noticed that high CRP level is associated with highly positive aCL [28,29] antibody, therefore this marker could be considered as an important acute phase reactant to APLA.

D-dimer reaction was significantly important in moderately and highly positive APLA. Indeed most patients with recent cerebrovascular thrombosis CVT, D-dimer concentration were increased & negative D-dimer assay may make the diagnosis of CVT very unlikely(30). Elevation of D-dimer in antiphospholipid syndrome had been suspected [31]. The increasing use of the D-dimer assay in clinical practice could be extended to patients presenting with acute cerebrovascular ischemic events to help predict stroke subtype[32].

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پوخته

ئەنتیفوسفولینید ئەنتی بەدیس ل دەف نەخوشـێن گـەنجـێن توشبوێن جەلتا مەژی

پێشەکی و ئارمانج: ئارمانجا ڤێ ڤەکولینێ هەلسەنگاندنا رولێ ئەنتیفوسفولینید ئەنتی بەدی ل دەف نەخوشێن گەنج و توشبوییّن جەلتا مـەژی ودەستنیشـانکرنا جـورێن ئایزوتـایپێن کارتێکـەر یـێن چـەند ئەنتیفوسـفولینید گـرنگ وەکـو لوپـاس ئـەنتی کوئەگیولانـد، ئـەنتی کاردیولیپین، ئەنتی فوسفاتایدێت سیلینی.

ڕێکێن ڤەکولینێ: ئەو ڤەکولینا ژجورێ (نەخوش و کونترول) هاتە ئەنجامدان ل نەخوشخانا ئبن سینا و ئەلسـەلام یـێن فێرکرنـێ. (75) نەخوشێن گەنج (ژیێ وان ژ45 سالیێ کێمتر) بو توشبویێ جەلتا مەژی وهاتیـە دەستنیشـانکرن برێکـا (MRI) هاتنـە ژێکـرتن بـو ڤـێ ڤەکولینێ. دەستنیشانکرنا ئەنتی فوسفولیتید ئەنتی بەدی هاتەکرن بو لوپاس ئەنتی کایگرنت و ئەنتی کاردولیپین و ئەنتی فوسفاتیدێت سیلینی ب رێکا (Immunoassay). ئەنتی نیوکلیر ئەنتی بەدیس، پروتێن جـورێ سـیری ئەکتـد ودی دایمـار هاتـە پشـکنین کـرن ب رێکێن ئمیونو سیرولوجی. نمونەك ژ(50) مروڤێن ساخ و سەلیم ب هەمان تەمەن و رەگەز هاتنە ژێگرتن وەك گروپێ کونترولی.

ئـەنجام: ئـەو نەخوشـێن هاتینـە دیـتن ب رێـژا (26.7٪) پـوزەتیڤ بـو ئـەنتی بـەدیا و ب تەمـەنێ رێـژا (32) پلاماینـەس (12) سـال وهەروەسا ب رێژا (2) بێت بو (3) بێت مێ. لوپاس ئەنتی گایدیولانت هاتە دیتن ژ(8٪) ژڤان حالەتاندا. ئەنتی کاردیولیپینی ئەنتی بەدی ب رێژەیەکا بەرچاڤ پتر هاتە دیتن لدەف نەخوشێن توشی جەلتا مەژی بوین بەراورد گروپێ کونترولی ($P < 0.001$) بو هـەر جـورەکێ ئەنتی بەدیا. هەروەسا رێژا ئەنتی فوسفاتیدێن سیلینی (IgG IgM) ئەوژی ب رێژەیەکا بەرچاڤ زێـدەتربون دگـەل نەخوشـا بـەراوەرد دگەل گروپێ کونترولی ($P < 0.001$) رێژێن کێم هاتنە دیتن بو هەردو ئەنتی کاردیولیپین و ئەنتی فوسفاتیدینێ سیلینی بێت کێم و بێ قیمەت لدەف نەخوشێن گەنج یێن توشبوی جەلتا مەژی. رێژا (CPR) یا بلندبو ب شێوەکێ بەرچاڤ لدەف نەخوشێن گـەنج توشـبوییّن جەلتا مەژی ($P < 0.05$). هەروەسا دیدایمەر یێ پوزەتیڤ بو لدەف نەخوشێن ئەنتی فوسفولیپید هەی ($P < 0.001$).

دەرئەنجام: رێژێن نافنجی و یێن بلند ژئەنتی فوسفولیبید ئەنتی بەدی هاتنە دیتن کو رولەکێ کارتێکەر یێ هەی لسـەر توشـبوونا جـەلتا مەژی دەف نەخوشێن گەنج یێن تەمەنێ وان ژ(45) کێمتر.

الخلاصة

تقييم ضد الفوسفوليد في المرضى الشباب الذين يعانون من سكتة دماغية

الاهداف: لتقييم ضد الفوسفولبد في المرضى الشباب الذين يعانون من سكتة دماغية نتيجة انصمام خثري. ولمعرفة طبيعة ونوع هذا الضد مثل ذأب ضد التخثر/ ضد الفوسفوتيدايل سيرين وضد الكارديولبين وايضاً لدراسة معايير اخرى ليس لها علاقة بضد الفوسفولبد.

الطريقة: نوع الدراسة هي دراسة حالات ومقارنتها مع مجموعة ضابطة هذه الحالات كانت موجودة في مستشفى ابن سينا التعليمي ومستشفى السلام التعليمي، وكانت اعمارهم دون سن 45 سنة ولديهم سكتة دماغية (مشخصون بواسطة فحص الرنين المغناطيسي). تم ايجاد ضد الفوسفولبد عن طريق فحص ذاب ضد التخثر وقياس نسبة ضد الكارديولبين وضد الفسفوتيدايل سيرين بطريقة المقايسة المناعية. دراسة ضد النواة وبروتين الفعال سي و فحص دي المزدوج بطريقة مصلية مناعية.

النتائج: كانت الحالات الموجبة لضد الفسفولبد بنسبة (26.7٪) ومتوسط العمر كان 32 ± 12 سنة. نسبة الذكور الى الاناث 2 : 3 .

ذأب ضد التخثر كان موجباً في 8٪. الحالات الموجبة بنسبة عالية وبنسبة متوسطة لضد الكارديولبين وضد الفسفوتيدايل سيرين نوع IgG وIgM كانت لهم اهمية كبيرة مقارنة بالحالات الضابطة بقيمة $P<0.001$. بينما لا يوجد أي اهمية لقيمة ضد الفوسفولبد الذي كان موجباً بقيمة قليلة لعدم وجود فرق عن المجموعة الضابطة.

ارتفاع البروتين الفعال سي كان له اهمية في مثل هذه الحالات وبقيمة ($P<0.05$) وكان فحص دي المزدوج موجباً في كل هذه الحالات وذا اهمية بقيمة ($P<0.001$).

الخلاصة: الارتفاع المتوسط والعالي لقيمة ضد الفوسفولبد كان له اهمية كبيرة في حصول السكتة الدماغية الناتجة عن انصمام خثري في الاشخاص الذين كانت اعمارهم اقل من 45 سنة.

PROTECTIVE ROLE OF SOME ANTIOXIDANTS ON SPERM ABNORMALITIES CAUSED BY NICOTINE IN ALBINO MICE *MUS MUSCULUS*

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GALAWEZH O.OTHMAN **

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ABSTRACT

To study the preventive effect of both vitamin C (Ascorbic acid) and vitamin E (a- tocopherol) on the sperm abnormalities in laboratory albino mice *Mus musculus* nicotine was administrated using three concentrations (10,20 and 30 µg/ mouse) through four doses daily (from 7:00 am to 7:00 pm) and for three different periods (15,30 and 45 days) while vitamin C & E were administrated half hour before and after nicotine administration with dose (100 µg/ mouse & 0.6 I.U/mouse) respectively.
The following results were observed:
1-Ability of nicotine to increase all types of qualitative sperm abnormalities in male mice significantly at ($P \leq 0.01$).
2- Vitamin (C) showed a strong antagonized affect against nicotine, while vitamin (E) did not show a significant effect.

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Key words: Nicotine effect, sperm abnormalities, and antioxidant.

Smoking have a cytotoxic effect on spermatozoa by reducing their number and decreasing their ability to function [1] or it could lead to cytogenetic abnormalities or mutation in spermatozoa that inherited to off -spring, potentially resulting in adverse reproductive and developmental out come such as spontaneous abortion [2].

Many studies have been revealed the protective effects of antioxidant vitamins on the risk of anomalies caused by the damage of free radicals resulted from smoking [3]. Antioxidants reduce teratogenicity and sexual abnormality of germ cells also that caused by chemical and environmental factors [4].

The aim of the present study is to study the effect of nicotine on sperm and the antagonistic effect of antioxidant with vitamin on sperm abnormalities if happened.

METHODS

One hundred sixty five mature males mice weighing between (29-32 gm) were obtained from animal house of college of education- Salahaddin University- Erbil-Iraq. Hundred thirty five mice were treated with different concentration of nicotine (10,20 and 30 ug/ mouse) for three different periods(15,30 and 45 days) nicotine administrated four times daily from (7:00 am to 7:00 pm). Forty five mice treated with (100ug/ mouse) of vitamin C half hours before and after administration of nicotine while other forty five mice were treated with (0.6 I.U./mouse) vitamin E also half hours before and after administration the 15 other mice were administrated only (0.2ml) olive oil which considered as a control for (vitamin E and nicotine), the last 15 mice were maintained under normal condition with normal diet and drinking water which represent the control group for both (nicotine and nicotine with vitamin C) treatment.
Karanaweska(1976) method was used with slight modification to prepare sperm from epididymus and vas deferent. For

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statistical analysis of data the factorial experiment was used.

RESULTS

(Table 1) showed that nicotine (concentration, periods and their inter action) significantly could create all types of sperm abnormalities at (P<0,01) includes (sperm without tail (S.W.T.T), sperm without head (S.W.T.H), swollen head sperm (S.H.S.) defective hook sperm (B.HO.S.) as shown in (Figures 1,2 &3).

Table 1. Analysis of variance for the effect of nicotine (concentrations, periods and their Interaction) on sperm abnormalities in laboratory mice .

Abnormalities		EMS					
Source of variation	d.f	Sperm Without tail	Sperm without head	Swollen head sperm	Defective head sperm	Defective hook sperm	Blunt hook sperm
Replication	4	0.29	1.2	0.30	0.23	0.07	1.92
Concentration	3	** 1606	** 1366.3	** 705.5	** 164	** 75.3	** 65.6
Period	2	** 88.05	** 159.3	** 167.8	** 168.7	** 14.05	** 54
C,P Interaction	6	** 90.65	** 92.75	** 27.05	** 20.9	** 80.7	** 10.4
Error	44	0.32	1.25	0.39	0.42	0.13	0.57
Total	59	9.18	88.7	44.6	35.1	16.9	7.3

** (P < 0.05) ** (P < 0.01)*

While it was observed from (Table 2) that as the concentration of nicotine increased, the highest value was in (S.W.T) (23.46+1.090) .

.The results of vitamin E administration with nicotine showed that vitamin E could decrease the toxicity of nicotine concentration, eriods and their interaction showed highly significant effect on all types of sperm abnormalities like (S.W.T.T) (S.W.T.H) (S.H.S) (D.H.S) and (D.Ho.S) as shown in (Table 3) while (Ttable 4) showed mean + S.E for vitamin E and nicotine effect the highest value was found in third concentration in (S.W.T.). Ascorbic acid had a significant effect on minimizing the sperm abnormality as shown in (Table 5) that highly significant difference at (P<0.01) were found in all types of sperm abnormalities in cases of concentration, while periods show highly significant effect at (P<0.01) in both (S.W.T.) and (S.H.S). Whereas significant different at (P<0,01) was found only in (D.H.S) and non-significant effects on another types of sperm abnormalities showed.

Interaction between periods and concentration showed non-significant effect on all types of sperm abnormalities.

The means of sperm abnormalities in mice treated with both vitamin C and nicotine showed highest value found in (20 µg/ mouse) second concentration in case of (Sw.H.S) (6.60=0.283). While period showed the highest value in case of (S.H.S) (5.400+0.596) in third period.

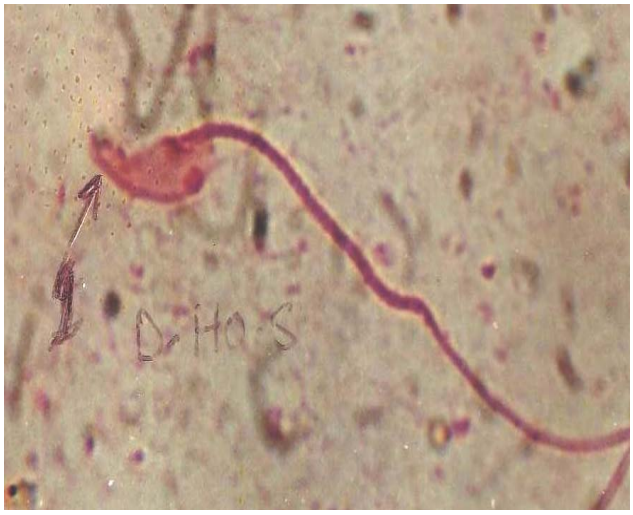

Figure 1. shows Defective hook sperm.

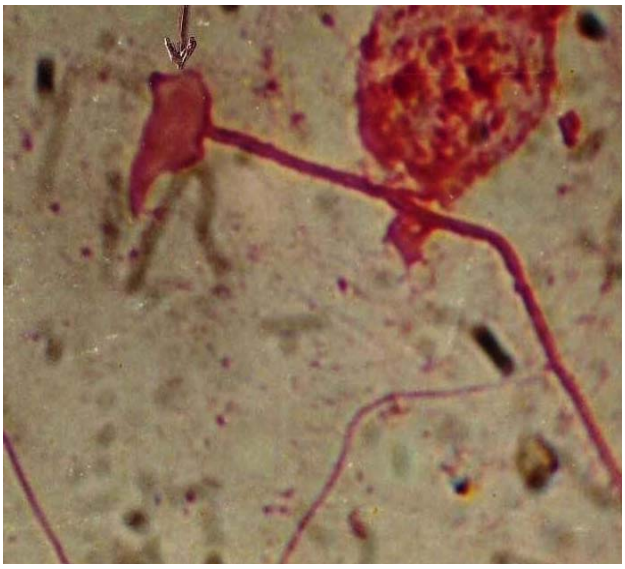

Figure 2. shows Defective head sperm.

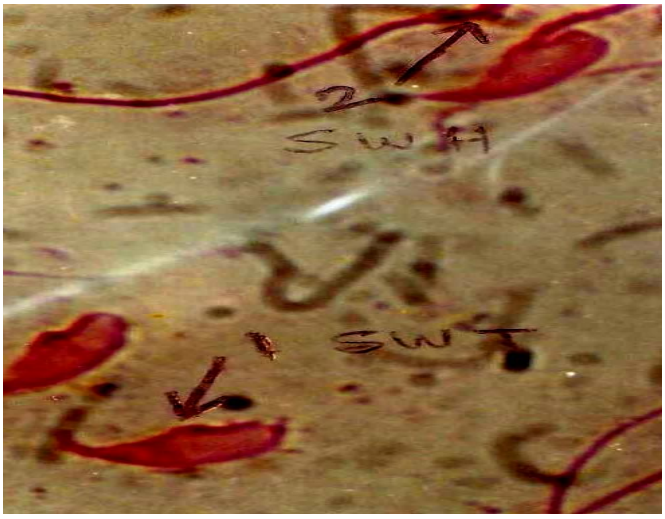

Figure 3. sperm without head, sperm without tail

Table 2. Mean ± S.E for the effects of nicotine (Concentrations, periods, and their interaction) on sperm abnormalities in Laboratory mice.

Abnormalities / Factors		Sperm with out tail	Sperm without head	Swollen head sperm	Defective head sperm	Defective hook sperm	Blunt hook sperm
Concentrations	C_0	0.266 ± 0.012	0.999 ± 0.001	0.300 ± 0.033	0.600 ± 0.001	0.366 ± 0.010	1.010 ± 0.067
	C_1	19.00 ± 1.693	16.866±0.559	10.266±0.948	10.375±0.898	5.460 ± 1.129	3.201 ± 0.321
	C_2	19.60 ± 2.236	21.066±1.420	14.416±0.695	13.73 ± 0.693	6.860 ± 0.798	4.360 ± 0.244
	C_3	23.46 ± 1.090	22.06 ± 0.520	15.06 ± 0.992	12.93 ± 0.371	7.330 ± 0.749	5.011 ± 0.340
	L.S.D	0.33	0.67	0.37	0.39	0.52	0.45
Periods	P_1	20.00 ± 1.407	16.266±0.308	17.53 ± 0.503	9.133 ± 0.631	5.860 ± 0.999	4.360 ± 0.342
	P_2	21.20 ± 0.637	18.533±0.884	9.933 ± 0.511	14.53 ± 0.133	5.530 ± 0.701	5.070 ± 0.348
	P_3	23.266±0.637	19.54 ± 1.858	18.630±0.625	13.733±0.529	8.620 ± 0.712	10.001±0.211
	L.S.D	0.29	0.20	0.31	0.33	0.18	0.38
Concentrations , Periods interactions	$C_0 P_1$	0.100 ± 0.082	0.600 ± 0.189	0.000 ± 0.000	0.200 ± 0.011	0.200 ± 0.013	0.400 ± 0.030
	$C_0 P_2$	0.000 ± 0.000	0.400 ± 0.109	0.400 ± 0.121	0.600 ± 0.211	0.400 ± 0.213	0.600 ± 0.030
	$C_0 P_3$	0.200 ± 0.000	0.600 ± 0.187	0.600 ± 0.230	0.100 ± 0.000	0.000 ± 0.000	0.600 ± 0.211
	$C_1 P_1$	20.399±0.240	16.60 ± 0.447	7.500 ± 0.258	8.000 ± 0.544	11.00 ± 0.316	2.012 ± 0.310
	$C_1 P_2$	24.80 ± 0.200	13.80 ± 0.374	8.000 ± 0.301	7.410 ± 0.243	2.600 ± 0.233	3.013 ± 0.410
	$C_1 P_3$	27.20 ± 0.663	17.40 ± 0.400	14.20 ± 0.240	11.601±0.244	2.810 ± 0.374	8.011 ± 0.300
	$C_2 P_1$	14.601±0.509	15.60 ± 0.374	12.60 ± 0.260	10.20 ± 0.200	3.180 ± 0.286	1.220 ± 0.410
	$C_2 P_2$	16.10 ± 0.412	20.60 ± 0.410	17.00 ± 0.632	15.00 ± 0.312	7.420 ± 0.244	3.211 ± 0.301
	$C_2 P_3$	24.81 ± 0.244	27.80 ± 0.280	19.60 ± 0.341	16.00 ± 0.316	12.00 ± 0.100	5.651 ± 0.311
	$C_3 P_1$	16.80 ± 0.200	16.201±0.670	10.40 ± 0.244	11.40 ± 0.240	4.000 ± 0.321	3.880 ± 0.412
	$C_3 P_2$	18.00 ± 0.447	18.00 ± 0.447	8.200 ± 0.347	13.41 ± 0.410	7.420 ± 0.221	2.710 ± 0.987
	$C_3 P_3$	25.805±0.670	25.80 ± 0.200	15.00 ± 0.227	14.201±0.200	10.62 ± 0.231	6.170 ± 0.871
	L.S.D	0.59	1.18	0.66	0.67	0.37	0.79

Table 3. Analysis of variance for the effect of Nicotine (concentrations, periods and their Interaction) on the sperm abnormalities of the laboratory mice after administrations of vitamin E. (before and after oral administration nicotine).

Abnormalities / Source of variation	d.f	Sperm without tail	Sperm without head	EMS Swollen head sperm	Defective head sperm	Defective hook sperm	Blunt hook sperm
Replication	4	1.35	0.72	0.087	0.39	0.275	0.482
Concentration	3	** 227.4	** 987.2	** 828.6	** 104.4	** 77.8	** 117.7
Period	2	** 111.2	** 437.6	** 151.6	** 49.8	** 65.5	** 42.35
C,P Interaction	6	** 39.4	** 69.2	** 98.8	** 8.38	** 17.0	** 11.6
Error	44	0.95	1.19	0.78	0.66	0.520	0.528
Total	59	47.13	73	57.9	8.37	115.931	90.2

** (P < 0.05) ** (P < 0.01)*

Table 4. Mean ± S.E for the effects of nicotine (Concentrations, periods, and their interaction) on sperm abnormalities in Laboratory mice after administration of vitamin E. (before and after oral administration of nicotine).

Abnormalities / Factors		Sperm with out tail	Sperm without head	Swollen head sperm	Defective head sperm	Defective hook sperm	Blunt hook sperm
Concentrations	C_0	2.400 ± 0.540	1.200 ± 0.316	0.989 ±0.100	1.600 ± 0.233	0.366 ± 0.010	1.200 ± 0.202
	C_1	13.00 ± 0.427	19.00 ± 0.639	11.80 ± 0.394	8.866 ± 0.145	7.400 ± 0.773	2.660 ± 0.351
	C_2	22.723±0.680	19.733±0.395	12.60 ± 0.216	9.330 ± 0.432	8.333 ± 0.582	4.676 ± 0.320
	C_3	24.001±0.673	20.336±0.757	12.989±0.833	10.333±1.290	9.466 ± 0.426	5.122 ± 0.185
	L.S.D	0.59	0.25	0.54	0.49	0.44	0.43
Periods	P_1	12.341 ± 0.331	15.112±0.660	10.712±0.662	7.811 ± 0.863	3.220 ± 0.720	2.636 ± 0.162
	P_2	10.620 ± 0.540	17.221±0.566	12.00 ± 0.412	5.660 ± 0.373	5.332 ± 0.750	4.200 ± 0.912
	P_3	24.660 ± 0.941	19.860±0.894	14.33 ± 0.598	8.660 ± 0.728	7.133 ± 0.646	6.712 ± 0.312
	L.S.D	0.50	0.18	0.45	0.42	0.37	0.370
Concentrations , Periods interactions	$C_0 P_1$	0.600 ± 0.109	0.400 ± 0.044	0.800 ± 0.133	1.000 ± 0.168	0.200 ± 0.099	0.400 ± 0.030
	$C_0 P_2$	0.400 ± 0.044	0.600 ± 0.100	0.600 ± 0.189	0.600 ± 0.070	0.600 ± 0.144	0.000 ± 0.000
	$C_0 P_3$	0.600 ± 0.100	0.200 ± 0.089	1.000 ± 0.238	0.600 ± 0.142	0.000 ± 0.000	0.800 ± 0.167
	$C_1 P_1$	10.80 ± 0.374	20.201±0.115	11.60 ± 0.927	12.00 ± 0.780	7.000 ± 0.282	5.000 ± 0.313
	$C_1 P_2$	11.40 ± 0.886	15.80 ± 0.373	4.800 ± 0.374	3.800 ± 0.630	8.800 ± 0.121	6.021 ± 0.112
	$C_1 P_3$	15.00 ± 0.663	21.002±0.440	17.00 ± 0.316	8.800 ± 0.372	4.200 ± 0.374	8.233 ± 0.201
	$C_2 P_1$	15.80 ± 0.374	12.00 ± 0.632	8.400 ± 0.117	7.401 ± 0.400	5.711 ± 0.221	4.890 ± 0.233
	$C_2 P_2$	20.41 ± 0.244	17.66 ± 0.261	4.400 ± 0.214	6.700 ± 0.512	7.610 ± 0.312	5.300 ± 0.112
	$C_2 P_3$	26.60 ± 0.509	24.00 ± 0.441	11.800±0.374	8.400 ± 0.400	10.001±0.316	7.022 ± 0.521
	$C_3 P_1$	20.33 ± 0.561	16.512±0.781	12.671±0.374	9.811 ± 0.669	4.801 ± 0.140	3.610 ± 0.231
	$C_3 P_2$	21.50 ± 0.310	18.33 ±0.750	14.811±0.128	10.72 ± 0.541	5.111 ± 0.220	4.711 ± 0.620
	$C_3 P_3$	25.70 ± 0.310	27.60 ± 0.032	16.861±0.012	12.66 ± 0.330	8.201 ± 0.312	6.210 ± 0.340
	L.S.D	1.03	0.93	0.92	0.86	0.76	0.76

Table 5. Analysis of variance for the effect of nicotine (concentrations, periods and their Interaction) on the sperm abnormalities of the laboratory Mice after administration of vitamin C. (before and after oral administration of nicotine).

Abnormalities / Source of variation	d. f	EMS Sperm without tail	Sperm without head	Swollen head sperm	Defective head sperm	Defective hook sperm	Blunt hook sperm
Replication	4	0.05	1.4	0.85	0.45	0.19	0.29
Concentration	3	** 1.04	* * 13.3	** 12.9	** 21.2	** 21.3	** 10.5
Period	2	** 2.7	0.72	** 5.1	* 4.3	0.12	3.9
C,P Interaction	6	1.3	2.6	1.6	2	1.55	3.31
Error	44	0.40	1.9	0.8	0.91	0.94	1.45
Total	59	1.6	3.1	1.9	6.2	7.6	7.6

**(P < 0.05) ** (P < 0.01).*

Table 6. Mean ± S.E for the effects of nicotine (Concentrations, periods, and their interaction) on sperm abnormalities in Laboratory Mice after administration of vitamin C. (before and after oral administration of nicotine).

Abnormalities / Factors		Sperm without tail	Sperm without head	Swollen head sperm	Defective head sperm	Defective hook sperm	Blunt hook sperm
Concentrations	C_0	0.266 ± 0.012	0.999 ± 0.001	0.300 ± 0.033	0.600 ± 0.001	0.366 ± 0.010	1.010 ± 0.067
	C_1	1.200 ± 0.193	2.666 ± 0.734	1.733 ± 0.312	1.660 ± 0.802	2.133 ± 0.321	1.211 ± 0.201
	C_2	2.210 ± 0.274	3.300 ± 0.905	2.500 ± 0.283	3.400 ± 0.420	3.500 ± 0.100	3.300 ± 0.101
	C_3	3.733 ± 0.893	6.600 ± 1.632	3.733 ± 0.247	2.000 ± 0.447	4.400 ± 0.541	2.841 ± 0.244
	L.S.D	0.38	0.58	0..54	0.58	0.59	0.73
Periods	P_1	3.200 ± 0.093	4.330 ± 0.876	4.860 ± 0.520	3.930 ± 0.973	1.100 ± 0.481	2.012 ± 0.344
	P_2	2.530 ± 0.093	2.200 ± 0.805	3.201 ± 0.470	1.660 ± 0.531	1.000 ± 0.020	2.200 ± 0.274
	P_3	3.860 ± 0.970	2.060 ± 1.380	5.400 ± 0.590	2.530 ± 0.702	1.686 ± 0.566	2.866 ± 0.451
	L.S.D	0.33	0.6	0.47	0.50		
Concentrations , Periods interactions	$C_0 P_1$	0.100 ± 0.082	0.600 ± 0.189	0.000 ± 0.000	0.200 ± 0.011	0.200 ± 0.013	0.400 ± 0.030
	$C_0 P_2$	0.000 ± 0.000	0.400 ± 0.109	0.400 ± 0.121	0.600 ± 0.211	0.400 ± 0.213	0.600 ± 0.030
	$C_0 P_3$	0.200 ± 0.000	0.600 ± 0.187	0.600 ± 0.230	0.100 ± 0.000	0.000 ± 0.000	0.600 ± 0.211
	$C_1 P_1$	1.600 ± 0.678	6.600 ± 0.509	2.200 ± 0.244	1.200 ± 0.370	1.100 ± 0.182	2.100 ± 0.271
	$C_1 P_2$	1.200 ± 0.660	1.200 ± 0.800	3.000 ± 0.075	1.600 ± 0.433	2.180 ± 0.374	2.600 ± 0.107
	$C_1 P_3$	1.000 ± 0.244	1.000 ± 0.300	2.200 ± 0.374	1.000 ± 0.316	6.600 ± 0.244	1.201 ± 1.007
	$C_2 P_1$	1.800 ± 0.425	1.600 ± 0.509	2.400 ± 0.123	2.400 ± 0.248	2.000 ± 0.000	2.908 ± 0.100
	$C_2 P_2$	2.410 ± 0.050	2.200 ± 0.140	3.400 ± 0.447	2.400 ± 0.401	1.600 ± 0.244	3.301 ± 0.202
	$C_2 P_3$	3.000 ± 0.836	1.600 ± 0.316	3.205 ± 0.374	1.500 ± 0.244	2.000 ± 0.244	2.205 ± 0.621
	$C_3 P_1$	1.400 ± 0.374	2.200 ± 0.244	4.200 ± 0.347	1.750 ± 0.428	1.023 ± 0.299	1.200 ± 0.502
	$C_3 P_2$	2.220 ± 0.142	2.000 ± 0.401	2.160 ± 0.583	2.200 ± 0.310	2.210 ± 0.237	3.110 ± 0.260
	$C_3 P_3$	2.000± 0.140	2.410 ± 0.240	3.000 ± 0.140	1.310 ± 0.347	1.660 ± 0.231	2.990 ± 0.102
	L.S.D	0.67	0.46				

DISCUSSION

Statistically, a highly significant effects of nicotine on the qualitative characteristic of sperm was observed. There are two different mechanism by which the nicotine cause sperm abnormalities, the first mechanism is an ability of nicotine to cause the cellular oxidative damage to the precursor cells, primary and secondary spermatocyte, that nicotine may lead to DNA damage and alternation of sperm formation (formation of abnormal sperm)[6] this clarification was supported by [7] which they observed that may lead to birth defect, and genetic disease in the off springs, also[8] showed that cigarette smoking could have a cytotoxic effects on spermatozoa by reducing the number the number sperm abnormalities leading to cytogenetic abnormalities that are passed to the offspring [1].

The second mechanism is the effect of nicotine on the endocrine function of the male reproduction system. Nicotine explores can causes reduction of secretion of testosterone hormone [9]. This inhibition leads to the arresting of the process of Spermatogenesis and unaffecting spermatogenesis which lead the formation of abnormal sperm with reduction of the number of the spermatids [10]. This was supported by [11] from their study on the (24) albino mice observed that nicotine caused a fail in spermatogonia and primary

spermatocytes number in the treated animals together with increase of spermatocytes, spermatid development process, they also observed that sertoli cells seems to be not affected by Nicotine administration in the doses equivalent to 10,20 except on day (14) of third dose equivalent to 30 cigarettes treatment. It seem likely that vitamins causes alterations in testicular DNA that disrupts the process of differentiation of sperm during spermatogenesis resulting in induced sperm abnormalities [12], described sperm abnormalities as a consequences of chromosomal aberrations and late change in the genes responsible for spermatogenesis.

While results of nicotine with vitamin C showed that this water soluble vitamin have ability to cause significantly reduction in the qualitative sperm abnormalities created by nicotine toxicity, these results were supported by [13] who administrates vitamin C with nicotine and he cocluded that teratogenic effect of nicotine was reduced by ascorbic acid and he returned the preventative effect of vitamin to its ability to alter the metabolic pathway of nicotine which lead to reduction of its toxicity.

The reduction through using β-tocopherol is may be due to its antioxidant effect, which inhibit the genotoxicity of nicotine on the testicular cells [14] and this was supported by [15] a study conducted that vitamin E supplementation can reduce the amount of sperm abnormalities caused by toxic effect of sodium fluoride on the reproductive organ of male mice.

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پوخته

لە کاریگەری بازدانی نیکۆتین وتاقی کردنەوەی هەندێك لە دژە ئۆکسانەکان (ڤیتامینەکان) دەکات لەسەر نێرەی مشکی سپی تاقیگەیی *Mus musculus* لەجۆری Balb/c،

ئەم توێژینەوەیە باس لە کاریگەری بازدانی نیکۆتین وتاقی کردنەوەی هەندێك لە دژە ئۆکسانەکان (ڤیتامینەکان) دەکات لەسەر نێرەی مشکی سپی تاقیگەیی ***Mus musculus*** لەجۆری Balb/c، بە بەکار هێنانی سێ خەستی جیاوازی نیکۆتین (10،20وە 30 مکگم) وە ئەم خەستیانە درا بە چوار بر لە رۆژێکدا لە کاتژمێر (7)ی بەیانی تاوەکو کاتژمێر(7)ی ئێوارەوە بۆ سێ ماوەی جیاواز (15،30وە45) رۆژ. بەڵام ڤیتامینەکان (E ,وەC) بە خەستی (، 0.6 یەکەی دەولی وە 100 مکگم) یەك بە دوای یەك . پێی درا نیو سەعات پێش وپاشی پێدانی نیکۆتین ، وە ئەم تاقی کردنەوەیە بەردەوام بوو بۆ ماوەی (15،30وە45) رۆژ ، وە ئەمانەی خوارەوە دیاری کران. :

1) نیکۆتین توانای زیاد کردنی ناتەواوی لە تۆودا هەیەوەك تۆوی (بێکلك، بێسەر، سەر هەڵئاوساو، سەر تێکچوو، قولاب تێکچوو، قولاب چەماوە). ($P \geq 0.01$).
2) ڤیتامین C کاریگەریەکی پێچەوانەیبەهێزی هەبوو لەسەرکاریگەری بازدانی دزی نیکۆتین بەڵام ،ڤیتامین E کاریگەریەکی وا بە هێزی نەبوو.

الخلاصة

التأثيرات التطفيرية للنيكوتين و اختبار تأثير بعض مضادات الأكسدة (الفيتامينات) في ذكور الفئران البيضاءالمختبريه *Mus musculus* من ضرب *BALB/C*

تبحث الدراسة الحالية عن التأثيرات التطفيرية للنيكوتين و اختبار تأثير بعض مضادات الأكسدة (الفيتامينات) في ذكور الفئران البيضاءالمختبريه *Mus musculus* من ضرب *BALB/C*. وباستخدام ثلاثة تراكيز مختلفة من النيكوتين (10 ،20و30مكغم) وأعطيت هذه التراكيز بأربعة جرعات في اليوم ابتداءً من الساعة السابعة صباحاً وحتى السابعة مساءاً ولفترات مختلفة (15 ، 30 و 45) يوميا، بينما أعطيت الفيتامينات (A , Eو C) بتراكيز (200و.د.,0,6و.د. و100مكغم)على التوالي نصف ساعة قبل و بعد اعطاء النيكوتين ،وقد استمرت التجربة لمدة(15،30 و 45) يوما.

وتم استنتاج ما يلي:

1- قابلية النيكوتين على إحداث التشوهات في حيامن الفئران المختبرية و المتمثلة بـ(حيمن فاقد الذيل sperm without tail، حيمن فاقد الرأس sperm without head ، حيمن منتفخ الرأس Swollen head sperm ، حيمن محطم الرأس Defective head sperm، حيمن محطم كلاب Defective hook sperm و حيمن منحرف كلاب Blunt hook sperm). ($P \geq 0.01$).
2- وجد بان فيتامين (C) له تأثير مضاد على التأثيرات التطفيرية للنيكوتين, أما فيتامين (E) فلم يكن له تأثير قوى.

THE EFFECT OF A COATING MATERIAL ON THE MICROLEAKAGE OF TEMPORARY SOFT DENTURE LINING MATERIALS

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ABSTRACT

Aims: The aim of this study is to evaluate the effect of a prepared coating material (monopoly) on the microleakage at the interface of two temporary soft denture lining materials (Tru-soft and Bony plus) with the acrylic resin denture base.
Methods: Sixty specimens for microleakage were prepared, each lining material had thirty. The specimen was in a disc shape 30mm diameter and 4mm thickness (2mm for acrylic resin denture base part and 2mm for soft lining material part). Tru-soft and Bony plus lining materials were bonded to three groups of denture base surface treatments (untreated, sandblasted and monomer treated denture base). A prepared coating material (monopoly) was applied at the junction of the denture base with the soft lining material, the specimens were placed in 2% methylene blue dye for two immersion periods (one week and one month) and the microleakage degree was measured by a microscope.
Results: Results of each lining material were analyzed separately and showed that for the two lining materials, there was a significant difference ($p < 0.001$) between coated and uncoated groups in all denture base surface treatments and in the two periods of immersion.
Conclusions: The coating material was effective in decreasing microleakage between the soft lining materials and the denture base when compared with the uncoated groups of the temporary denture lining materials.

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Key words: Microleakage, soft lining materials, coating material.

Soft denture lining materials are used in cases of resorped mandibular alveolar bone, thin and non resilient mucosal tissue, maxillofacial defects and for patients who are unable to tolerate the heat polymerized acrylic denture base,[1-3] but these materials fail for many reasons including: hardening, water sorption, color changes, support of bacteria and candida, but the most serious problem is the failure of adhesion between the soft denture lining and the denture base [4,5].

Adhesion failure between two different materials creates a gap for the passage of bacteria, fluids, molecules or ions, which is known as a microleakage [6]. This problem can cause a separation of the soft denture lining material from the denture base, a harbor for bacteria and may promote staining [7].

Microleakage is an early sign of a weakened bond,[2] therefore many attempts were done to enhance the bond and to decrease the leakage. Using coating materials to enhance the longevity of soft denture lining materials was studied by many researchers [8-10]. In this study the effect a prepared coating material at the junction of the acrylic-based temporary soft denture lining material and the acrylic resin denture base was undertaken.

METHODS

Two soft denture lining materials (Tru-soft, self cured acrylic based, Bosworth, USA) and (Bony-Plus, self cured acrylic based, Lietchtenstein, Switzerland) were used, specimens of microleakage consisted of thirty specimens for each lining materials bonded to the prepared acrylic resin denture base, each specimen is a disc shape 30mm diameter and 4mm thickness (2mm acrylic resin part and 2mm soft lining material part). A clear

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heat cured acrylic resin denture base (Quayle Dental, England) was prepared by packing a dough acrylic into stone molds, processing and curing were carried out according to manufacturer instructions. The acrylic resin denture base specimens were divided into three groups before bonding to the soft liners, these groups were: untreated, sandblasted with 250 μm Aluminum oxide particles and monomer treated group in which the specimens of denture base were wetted by a cotton tipped applicator, saturated with methyl methacrylate monomer three times for 180 seconds.

Tru-soft and Bony plus soft lining materials were applied by using a split mold (4mm thickness and 30mm diameter). Pre-cured denture base specimens were placed in the mold first, and then the soft lining materials were added, the split mold was placed between two plates to extrude excessive material.

Monopoly, thin syrup like mixture of a semi-set methyl methacrylate resin was prepared by mixing heat-cured clear methyl methacrylate powder with auto polymerizing clear orthodontic methyl methacrylate liquid in proportion of one part powder to ten parts liquid. The powder and liquid were placed together in a glass beaker in (130°F) water bath and stirred for 8-10 minutes until the mixture started to thicken [8]. The coating was applied to the junction of the Tru-soft and Bony plus lining materials with the acrylic resin denture base by using a fine brush, the coating was allowed to dry for 4-5 minutes under a lamp of 60 Watt, this procedure was repeated until three coats have been placed and dried.The specimens were immersed suspended in methylene blue dye inside plastic containers in an incubator at 37°C and divided into two groups of immersion, one week and one month. At the end of each immersion period, the specimens were removed, washed, dried and sectioned into eight pieces by a diamond sectioning disc using portable engine hand piece (W &H Dental Werk, Austria). Microleakage values were measured by the linear penetration of methylene blue dye from the edge of the soft liner / denture base interface by the aid of a stereoscopic (Carl Zeiss, Germany) at 40X magnification and the dye penetration was recorded in millimeter.

RESULTS

The effect of coating on the microleakage of Tru-soft and Bony plus lining materials was analyzed for each immersion period separately by ANOVA and Duncan's multiple range test. (Tables 1&3) showed that there was a significant difference (P<0.001) between coated and uncoated groups in all denture base surface treatments at one week and at one month immersion periods for each material.

Table 1. (ANOVA) of microleakage in (mm) of coated and uncoated Tru-soft lining material after one week and one month storage.

	Source of variation	*Sum of square*	df	Mean square	F-value	p-value
One week storage	Between groups	0.234	5	0.04681	135.24	<0.001*
	Within groups	0.0083	24	0.00035	.	
	Total	0.242	29	.	.	
One month storage	Between groups	0.114	5	0.02279	324.87	<0.001*
	Within groups	0.0017	24	0.00007	.	
	Total	0.115	29	.	.	

*df = Degree of freedom *Significant difference*

In Tru-soft lining material, (Table 2) showed that the coated group had the lowest mean microleakage in the monomer treated denture base (0.019mm, 0.022mm), while the uncoated groups had the highest

values in untreated denture base (0.184mm, 0.216mm) at one week and one month respectively. Results of Bony-plus soft lining material (Table 4) showed that the lowest values of micro leakage in coated monomer treated denture base (0.017mm, 0.02mm) in one week and one month immersion periods respectively, while the highest values were observed in uncoated groups in the untreated denture base (0.178mm, 0.193mm) at one week and one month respectively.

Table 2. (DMRT) of microleakage in (mm) of coated and uncoated Tru-soft lining material after one week storage.

Surface treatment	Coating	N	Mean ± SD (mm)	DMRT groups*
After one week storage				
Untreated	Uncoated	5	0.184 ± 0.013	E
	Coated	5	0.025 ± 0.003	A
Sandblasted	Uncoated	5	0.170 ± 0.005	D
	Coated	5	0.035 ± 0.004	B
Monomer treated	Uncoated	5	0.141 ± 0.006	C
	Coated	5	0.019 ± 0.003	A
After one month storage				
Untreated	Uncoated	5	0.216 ± 0.042	B
	Coated	5	0.028 ± 0.003	A
Sandblasted	Uncoated	5	0.209 ± 0.014	B
	Coated	5	0.039 ± 0.003	A
Monomer treated	Uncoated	5	0.192 ± 0.011	B
	Coated	5	0.022 ± 0.003	A

DMRT = Duncan's multiple range test
N = Number of specimens
** = Different letters mean statistically significant difference*

Table 3. (ANOVA) of microleakage in (mm) of coated and uncoated Bony plus lining material after one week and one month storage.

	Source of variation	*Sum of square*	df	Mean square	F-value	p-value
One week storage	Between groups	0.15	5	0.02998	658.12	<0.001*
	Within groups	0.0011	24	0.00005	.	
	Total	0.151	29			
One month storage	Between groups	0.15	5	0.02998	658.12	<0.001*
	Within groups	0.0011	24	0.00005		
	Total	0.151	29			

df = Degree of freedom
**Significant difference*

Table 4. (DMRT) of microleakage in (mm) of coated and uncoated Bony plus lining material after one week storage.

Surface treatment	Coating	N	Mean ± SD (mm)	DMRT groups*
After one week storage				
Untreated	Uncoated	5	0.178 ± 0.010	E
	Coated	5	0.039 ± 0.003	B
Sandblasted	Uncoated	5	0.156 ± 0.010	D
	Coated	5	0.047 ± 0.003	B
Monomer treated	Uncoated	5	0.120 ± 0.015	C
	Coated	5	0.017 ± 0.001	A
After one month storage				
Untreated	Uncoated	5	0.193 ± 0.005	F
	Coated	5	0.041 ± 0.003	B
Sandblasted	Uncoated	5	0.183 ± 0.003	E
	Coated	5	0.049 ± 0.007	C
Monomer treated	Uncoated	5	0.158 ± 0.010	D
	Coated	5	0.020 ± 0.003	A

DMRT = Duncan's multiple range test
N = Number of specimens
** = Different letters mean statistically significant difference*

DISCUSSION

Complete prevention of microleakage was not possible; However, many attempts to extend the longevity of a soft lining and decreasing microleakage were conducted like denture base surface treatments [2,11]. In the present study, two groups (with and without coating) of self-cured acrylic-based soft denture lining materials (Tru-soft and Bony plus) were employed for detecting the effect of a coating material on microleakage values. [1]

Although the amount of the coating material (monopoly) that was used to seal the denture base/ soft lining interface may vaporize during the drying by light, results of both soft lining materials showed that microleakage values were maintained at minimum levels when the coating material was applied than when the interface of the lining and the denture base was left uncoated. These results were in accordance with Dominguez et al., [9], Gronet et al., [12,] Malmstrom et al., [10] and Murata et al., [13]. All of them revealed that the coating material significantly reduced the loss of initial viscoelastic properties and surface integrity by acting as a barrier preventing movement of components to or from the lining material and decreased the incidence of bacterial and fungal growth for an extended period of time. Also the results agree with Anil et al., [14] who concluded that coating the soft lining materials is beneficial in reducing microleakage at the interface between the lining material and the acrylic resin denture base.

A possible explanation of this reduction in microleakage after the application of a coating material at the interface of the lining and the denture base is that the temporary acrylic-based soft lining materials contain appropriate amount of plasticizers which are necessary to produce effective softness and movement between the polymer chains of the lining, but these plasticizers readily leach out in aqueous

environment and the microbes and saliva on the other hand are absorbed by the porosities of the material causing degradation of the bonding interface and microleakage; consequently, the lining cease to be effective and should be replaced. By adding a coating material to seal the gap between the lining and the denture base, absorption and solubility of the lining material which is a contributing factor to the hardening and bond loss will be reduced because the porosities in the lining are obstructed. Eventually microleakage is decreased [9, 10] .

CONCLUSIONS

Application of the coating material on the denture base/ soft denture lining interface is significantly beneficial in reducing microleakage for the acrylic based temporary soft lining materials (Tru-soft and Bony plus).

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پوخته

كارتێكرنا كەرەستێ ب سەرڤە ژبەرهاتنا بەتەنا ددانا یا نەرم و یا بەروەخت

ئارمانج: ئارمانجا ڤێ ڤەكولینێ هەلسەنگاندنا كارتێكرنا بەتەنەكا دروستكری سەر ژبەرهاتنێ لسەر خالا گەهاندنێ یا دوو بەتەنێن ددانا بێن نەرم و بەروەخت ب رێكا بكارئینانا ئەكریلیك ریزین وەك بناغە بو ددانی.

رێكێن ڤەكولینێ: (60) نمونێن ژبەرهاتنێ هاتنە ئامادەكرن ئەو نموونە هەر ئێك ژوان شێوەیەكێ گروڤر كو (30)مم بو و ستویراتی (4)مم هەر دوو بەتەنێن نەرم و بەروەخت هاتبوونە گرێدان دگەل گروپێن ددانا ئەوێن بناغێت وا هاتینە چارەسەركرن ب ساند بلاستد و مونومەر. بەتەنەكێ ئامادەكری هاتە دانان لسەر جهێ تێكراچوونا بناغێ ددانی دگەل تەخا ددانی یا نەرم، ئەو نموونە هەروەسا هاتبوونە دانان (2٪) ژ میسیلینێ بلو بو دوو جارا حەفتیەك و هەیڤەك دناڤبەرا هەر جارەكێ و رێژا ژبەرچوونێ ب رێكا میكروسكوپێ هاتە هەژمارتن.

ئەنجام: ئەنجامێن هەر بەتەنەكی هاتنە شلوڤەكرن جوداجودا ودیاربو كو جیاوازیەكا بەرچاڤ ($P < 0.001$) دناڤبەرا گروپێن بناغێن ددانا ئەوێن هاتینە نخافتن و نەهاتینە نخافتن.

دەرئەنجام: كەرەستا نخافتنێ یا كاریگەر بو ل كێمكرنا رێژا ژبەرچوونێ دناڤبەرا بەتەنا نەرم یا نخافتنێ و بناغێ ددانی ل دەمێ بەراوەردكرنا ڤی كاری دگەل گروپێ نەیێ نخافتی ژبنەغایێن ددانێن بەروەخت.

الخلاصة

تأثير مادة طلائية على التسرب الدقيق لمواد بطانة طقم الأسنان الطرية المؤقتة

الهدف: تقييم تأثير مادة الطلاء المحضرة (mono-poly) على التسرب الدقيق بين مادتين من بطانة طقم الأسنان المرنة (Tru-soft and Bony plus) مع قاعدة الطقم من الراتنج الأكريلي.

المواد وطرق العمل: تم تحضير 60 عينة لقياس التسرب الدقيق، لكل مادة من بطانة الطقم 30 عينة. كانت العينة بشكل قرص بقطر 30ملم و سمك 4 ملم (2 ملم لجزء قاعدة الطقم الاكريلي و2 ملم لجزء مادة بطانة الطقم الطرية). تم ربط مادتي (Tru-soft and Bony plus) مع ثلاث مجاميع من معالجات سطح قاعدة الطقم (السطح غير المحضر، السطح المعالج بالنفخ الرملي والسطح المعالج بمحلول قاعدة الطقم). تم إضافة المادة الطلائية المحضرة (mono-poly) عند منطقة اتصال قاعدة الطقم مع مادة بطانة الطقم المرنة. وضعت العينات في (2٪) من صبغة الميثيلين الأزرق لفترتين من الغمر (أسبوع واحد وشهر واحد) وتم قياس درجة التسرب الدقيق بواسطة المجهر المدرج.

النتائج: بينت النتائج لكلا مادتي الطقم الطرية وجود تغير معنوي ($p <0.001$) بين المجاميع التي تم طلاؤها والتي تركت بدون طلاء في كل مجاميع معالجات سطح قاعدة الطقم وفي فترتي الغمر.

الاستنتاجات: كانت مادة الطلاء فعالة في خفض التسرب الدقيق بين مواد بطانة الطقم الطرية و قاعدة طقم الأسنان من الراتنج الأكريلي عند مقارنتها مع المجاميع التي لم يتم طلاؤها.

LAPAROSCOPIC ABLATION OF SYMPTOMATIC SIMPLE RENAL CYSTS AT AZADI TEACHING HOSPITAL (OUR INITIAL EXPERIENCE)

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ABSTRACT

Objective: To evaluate our experience with the use of laparoscopy in the management of patients with symptomatic renal cysts regarding out comes and to report any undesirable complication that may occur (if any) during and/or after this procedure.
Background: Renal cystic disease is a common finding in older persons, which may be discovered either incidentally or radiographically. Most of renal cystic diseases are asymptomatic, in a small number symptoms or complications develops, in whom laparoscopic surgery either trans- or retroperitoneally is now an option.
Methods: From March 2010 to July 2011, a total of 33 patients with symptomatic renal cysts underwent laparoscopic ablation (decortications) for their cysts in Azadi teaching hospital. Clinical assessment, operative details and complication were reported for all the patients.
Results: Laparoscopic ablation of renal cysts was successful in all patients. No open conversions or transfusions were necessary. The mean operative time was 53 minutes. The mean hospital stay was 1 day. Symptomatic relief was achieved in all patients. Only 2 patients developed minor complications which had been managed conservatively. No evidence of recurrence at the end of 6 months follow-up period.
Conclusion: Our results have confirmed that laparoscopy is a safe, feasible and effective procedure for treating symptomatic renal cysts, and it offers a favorable minimally invasive treatment option for symptomatic renal cysts.

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Key words: Laparoscopic, renal cyst, Azadi.

The use of laparoscopic surgery has rapidly expanded since the laparoscope merged with the video camera in the mid-1980s. Since then, significant developments of laparoscopic equipment and instruments have been made.[1]

The development of laparoscopy in urology paralleled, to a large extent, the changes in general surgery. Up until the late 1980s, laparoscopy had limited applications in urology. Indeed, aside from Cortesi and colleagues' (1976) report of using the laparoscope in pediatric patients to explore for undescended testes[3]. Clayman and coworkers (1991b) performed the first clinical laparoscopic nephrectomy[4].

With the development of new techniques and instruments a new era in operative urology had begun. Soon, a steady stream of newly developed laparoscopic procedures started to challenge their conventional open surgical counterparts and the Initial emphasis was on ablative procedures[5.]

With an increasing number of multi-institutional studies emerging in which laparoscopic procedures are compared with their open surgical counterparts, it becomes clear that, owing to equivalent efficacy combined with distinct advantages in postoperative pain, cosmesis, recovery, and length of hospital stay, that laparoscopy has moved into the mainstream of urologic surgery[6] .

Renal cysts are believed to develop in tubular segments and Bowman's capsule, with simple cysts developing from the convoluted tubule, whereas the acquired

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and genetic forms may develop anywhere along the nephron and collecting duct [7]. Most are unilocular and, because they arise from the cortex, they distort the normal renal contour. They may enlarge somewhat with time, although most probably remain stable in size and appearance.

The prevalence of simple cysts increases with age, and most reports show no gender predilection [8].

Most renal cystic diseases are asymptomatic, in small number symptoms or complications develop.

Patients with symptomatic renal cysts may be candidates for cyst aspiration under CT or ultrasound guidance but such cysts will almost certainly recur, it is reasonable to conclude that if the patient's pain resolves after cyst aspiration, then some definitive form of therapy may be warranted and justifiable [9].

The use of percutaneously injected sclerosing solutions has limited value. Open surgical approaches have been favored in the past [9], but the potential morbidity of the procedure must be weighed against the benefits. A minimally invasive approach including laparoscopic ablation offers a reasonable alternative [9].

The aim of this study was to show the efficacy of laparoscopy for managing patients with symptomatic renal cysts and to report any undesirable complication that may occur (if any) during and/or after this procedure.

METHODS

This prospective study was conducted in the department of urology at Azadi teaching hospital in Duhok/Iraq from March 2010 to July 2011.

During this period, all patients who were presented with symptomatic renal cysts and managed laparascopically had been enrolled in this study and a total of 33 patients (12 male and 21 female) were included in this study.

For each patient a complet evaluation has been done including:

A detailed medical history and physical examination.

Basic laboratory investigations including a urine analysis, Full blood count, biochemical investigations including Blood Sugar, blood urea and serum Creatinine levels were done for all patients.

Radiological examination including Ultrasound examination and CT examination were done for all patients to assess the type of the cyst, size of cysts, numbers of cysts, and their relationship to the adjacent organs and to exclude any associated pathology.

The entire procedure was performed in the operation theater (by tow urologists for all patients) with the patient under general anesthesia and patients placed in a 45 modified lateral flank position (Transperitoneal approach).

After establishment of pneumoperitoneum and insertion of ports (one 10mm & tow 5mm ports were used in the majority of patients, a fourth port was needed in few patients) the cyst then deroofed with laparoscopic scissors and its walls were carefully inspected to rule out malignancy. Then the edges of the cyst were carefully cauterized to minimize the chance of bleeding & recurrence.

After recovery from general anesthesia patients were transferred to the ward for observation for the next 24 hours and if everything was normal the patient was discharged from hospital in the next day.

All patients were followed up by Ultrasound examination of the abdomen at 3 months & six months for any recurrence.

RESULTS

During study period Laparoscopic decortications was performed for 33 patients. The age of the 33 enrolled patients as shown in(Figure 1) ranged from 37 years to 73 years, with mean age of 52.6 ± 9.13 years. Of these 33 patients 21 (63.7%) were female and the remaining 12 (36.3%) patients were male.

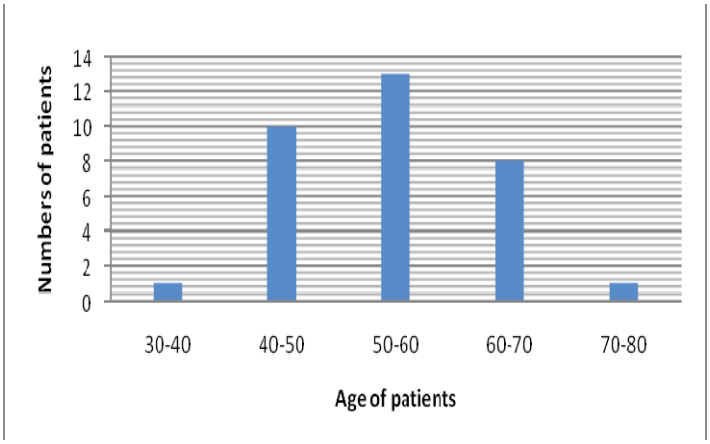

Figure 1. Age distribution of the all enrolled patients with renal cysts.

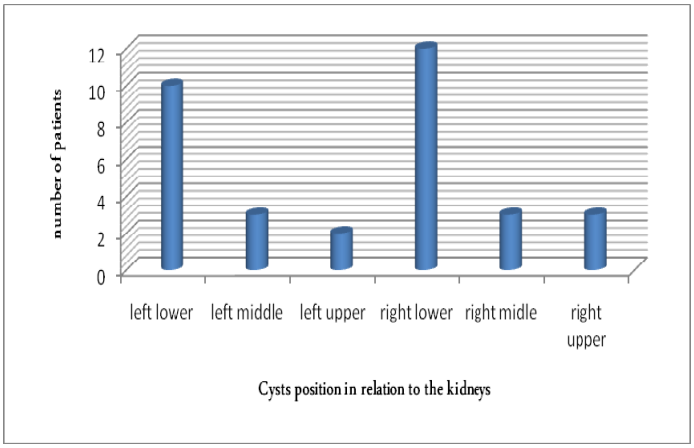

Figure 2. renal cyst position.

The presentation of all patients was similar and was loin pain and only 2 patients had also fever in addition to loin pain which has been managed with antibiotitics prior to the laparoscopy procedure.

Renal cysts were more frequently observed in the right side 54.6% (18 patients) in

Single cyst was seen in 84.8% (28 patients). In 3 (9.1%) patients there were 3 cysts (one is large that necessitate surgical intervention and the other 2 cysts were very small and did not need any intervention) and the remaining 2 (6.1%) patients had 2 renal cysts (one is large and the other was small). Also one female patient had bilateral ovarian cysts in addition to the renal cyst.

Renal cyst size was variable ranging from (40*40) mm to (103*109) mm.

Of the 33 enrolled patients, 12 of them had previous abdominal operation for various reasons, and even one of the female patients had 4 previous Cesarean section.

comparison to the left side 45.4% (15 patients).

As is shown in (Figure 2) renal cysts were observed to be more common in relation to the lower pole of the kidney 66.7% (22 patients) than to upper 18.2% (6 patients) and mid poles 15.1% (5 patients).

One patient has recurrent renal cyst, he had underwent cyst aspiration under U/S guide one month before laparoscopic intervention without any benefit (re-accumulation of fluid in the cyst).

The operation time (from the induction of anesthesia to the closure of the trochar sites) ranged from 40 minutes to 80 minutes, with a median time of 55 minutes (mean 55 ± 10.16).

Most of the procedures were performed using 3 ports only, of the 33 enrolled patients, only 7 of them needed placement of a 4th port during the operation for retractor placement.Drain had been put for 11 patients, which has been removed in the

next day for all patients except one who developed intra-operative bleeding and his drain has been removed after 2 days.

Two patients (6.1%) developed complication intra-operatively, one of them developed mild liver contusion and was managed conservatively, by increasing carbon dioxide insufflations (intra-operatively), tube drain and observation & after 24 hours the oozing completely stopped. Other patient developed intra-operative bleeding from the edge of the cyst & was managed by suturing of the edge of the cyst and tube drainage which was kept for 48 hours.

All patients had been discharged from the hospital in the next day except one who developed intra-operative bleeding and had been discharged from hospital after 2 days after removal of the drain.

The result of histopathologyical examination of the cyst wall was negative for malignancy in all patients.

DISCUSSION

Although the foundation of modern laparoscopy was laid in 1805 by Bozzini (Bozzini, 1806) [10], laparoscopy was not used widely in surgery till mid-1980s when it has been merged with the video camera[1].

From that time, laparoscopy was used widely in surgery including urology [2]. As urologists became more skilled, they expanded their laparoscopic procedures into the realm of more difficult ablative surgery[10].

This study has been conducted in our center in order to evaluate our laparoscopic experience in treating renal cysts.

Renal cystic disease generally is the ailment of older patients and this was observed in the present study were the mean age of patients was 54 years old.

Most of the patients were females and this may be due to low pain threshold of females that bring the cysts under clinical observation and diagnosis as the pain was the chief presentation of the renal cysts.

This study also revealed that most cysts were arising from the lower pole of the kidney (both from the right and left sides) and this may be related to the drainage of the lower poles in contrast to the mid and upper poles.

Laparoscopic interference was possible even in those with previous abdominal operation (even if multiple) as 12 out of 33 of our patients had previous abdominal operation.

The complication rates for urologic laparoscopy were varied significantly from one series to another ranging from 4.4% to 19%. Which may be due to variable opinions as to what constitutes a complication and differing degrees of technical difficulty between laparoscopic procedure [11]. In our study the complication rate was 6.1% (2 out of 33 patients) and these complications were mostly bleeding, as the most common complications of laparoscopic interference are vascular [11], and both of our patients developed oozing intraoperatively and had been managed conservatively by increasing carbon dioxide insufflations pressure (intra-operatively), intraperitoneal drainage via a tube drain & monitoring for one to two days.

Almost all renal cysts recur after aspiration and need more definite procedure for complete resolution as it has been seen in one patient who has been previously managed by aspiration under U/S guide and recur after 1 month & so laparoscopic decortications was done for him.

The operative time varies from one patient to other depending on the size & constituent of the cyst & surgeon experience, in our study the mean operation time was 55 minutes which is comparable to most reported series 12-15.

The recurrence rate was zero as no patient developed recurrence of cyst as documented twice post-operatively at 3months and 6 months post-operatively by ultrasound and renal function tests. These results were comparable to most reported series which give a nearly 100% success

rate for laparoscopic decortications of renal cysts 12-15.

CONCLUSIONS

Laparoscopy offers a safe and efficacious means of ablating symptomatic simple renal cysts with a high success rate and low recurrence rate.
Most complications that occur are simple and can be managed conservatively.

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پوخته

هەلگرتنی تویری سادە ل گورچیلە ب ڕێگای بکارهینانی ئامیری لاپروسکوب ل نەخوشخانە ئازادی فیرکردن

ئارمانج: ئەم توژینەوە هایتە کردن بو هەلسانگاندنی بکارهینانی ئامیری لاپروسکوب بو هەلگرتنی تویری سادە ل گەورچیلە ودیارکرنی هەموو ئو رووداوانەی نە دروست ل کاتی و ل پشت ئەنجامدانی ئەم جورە نشتەگەریە.

تویری سادەی گورچیلە جورە حالەتکە بەربەلاوە ل مروڤی بەتەمەن . دەست نیشانکردنی ئەم حالە تە دیتـە کـردن ل کـاتی وەرگرتنـی تشك بو هەر هویەکی دی یان ل کاتی دیاربوونی هندەك نیشان یان ئازار.ول کاتی دیاربوونی ئازار ,

ڕێکێن ڤەکولینێ: ئەم تویرە ب دیتە هەلگرتن ب ریگای لاپروسکوب .

ل ماوەی ئاداری 2010 و گەلاویژی 2011, (33) نەخوش تووشی تویری سادەی گورجیلە هاتینە چارەسەرکردن و تویرەکان هاتیە هەلگرتن ب ریگای بکارهینانی ئامیری لاپروسکوب ل نەخوشخانە ئازادی ل شاری دهوك.

ئەنجام:ل ئەنجامدا دیار کەوت کە, هەمەو نشتەگەریاکان سەرکەوتوو بو, هیچ پیویستی نەبوو بو کردنەوەی زگ یا دانانی خوین و ناوەند کاتی نشتەگەری (53) خولەك بوو و ناوەندی مانەوە نەخوشەکان ل نەخوشخانە یەك ڕۆژ بـوو. و دوو نـەخوش تووشـی هەنـد رووداوی سوك بوون وهاتینە چارەسەرکرن بی نشتەگەری. و هیچ نەخوش تووشی دووبارە دروست بوونی تویری سادە نەبون نەوە ل ماەی (6) مانگ پاش نیشتەگەری.

دەرئەنجام : ئەم توژینەوە دیاربو کە بکارئینانی ئامیری لاپروسکوب بو هەلگرتنی تویری سادەی گورچیلە باشترین وسلامەتین ریگایە.

الخلاصة

استئصال اكياس الكلى البسيطة بالمنظار في مستشفى آزادي التعليمي (خبرتنا الاولية)

الغرض: لتقييم نتائج تجربتنا لاستخدام منظار البطن في معالجة المرضى الذين يعانون من الاكياس الكلوية العرضية البسيطة ولبيان أي مضاعفات غير مرغوب فيها قد تحدث (إن وجدت) أثناء العملية أو بعدها.

الخلفية: مرض تكيس الكلى يعتبر تشخيصا شائعا لدى كبار السن، والتي قد يتم اكتشافها إما صدفة عن طريق الفحوصات الاشعاعية او نتيجة ظهور الاعراض. معظم أمراض تكيس الكلى عديمة الاعراض، في عدد قليل من المرضى تظهر الأعراض أو المضاعفات ، حيث تعد الجراحة بواسطة منظار البطن إما عبراو خلف الصفاق الان خيارا للعلاج.

الطرق: في الفترة من مارس 2010 الى يوليو 2011، خضع 33 مريضا يعانون من تكيسات كلوية عرضية لاستئصال الاكياس بالمنظار (decortications)في مستشفى آزادي التعليمي.

وقد دون التقييم السريري، تفاصيل العملية والمضاعفات لكافة المرضى.

النتائج: عملية استئصال الاكياس الكلوية بواسطة المنظار كانت ناجحة في جميع المرضى. حيث لم تستدعي الضرورة لعمليات فتح بطن او نقل الدم . وكان متوسط زمن العملية 53 دقيقة. و كان متوسط الإقامة في المستشفى يوما واحدا. حصلت مضاعفات بسيطة في مريضين و التي قد عولجت تحفظيا. لم تتواجد أدلة على تكرار التكيسات حتى نهاية فترة المتابعة(6 أشهر).

الاستنتاجات: أكدت نتائجنا أن تنظير البطن هو إجراء آمن ,مجدي وفعال في علاج تكيسات الكلى العرضية، وأنه يقدم خيار علاجيا ملائما و(minimally invasive) لتكيسات الكلى.

EFFECT OF APICAL PATENCY ON PERIAPICAL EXTRUSION DURING DIFFERENT INSTRUMENTATION TECHNIQUES

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ABSTRACT

Background and objectives: Establishing apical patency is leaving the apical foramen accessible, free from dentin chips, pulp fragments and other debris. This in vitro study compared the apical foramen patency for two different instrumentation techniques.
Methods: Thirty single-rooted human teeth were used in this study & the teeth were de-coronated at Cemento-enamel Junction. After that roots were fixed in holes cut through pieces of rubber dam covering plastic containers. The roots were divided into two groups, the first 15 were instrumented using a step-back technique & other 15were instrumented using crown-down technique. Both instrumentation techniques were used twice; once without a patency file & the other with a patency file. Finally the collection vials (the bottles) were weighed after instrumentation, i.e. with the irrigating solution (the amount of NaOCl + debris that was extruded through the apical foramen into the collection vials) on an electronic balance to the third decimal digit.
Results: By using t-test, there was non-significant difference between the Group A1 (step-back hand instrumentation without use of patency file) & A2 (step-back hand instrumentation with use of patency file) at $p > 0.05$, with in favor of without use of patency file group. There was highly significant difference between Group B1 (crown-down rotary instrumentation without use of patency file) & B2 (crown-down rotary instrumentation with use of patency file) at $p < 0.01$, with in favor of without use of patency file group. For the instrumentation techniques, the rotary crown-down technique showed lesser debris extrusion than the hand step-back technique both with & without use of patency file, however, the difference was non-significant at $p > 0.05$.
Conclusions: there is a strong relationship between the patency file & the extrusion of debris & the irrigation solution.

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Key words: Apical patency, periapical extrusion.

During root canal preparation, dentin chips produced by instrumentation and fragments of apical pulp tissue tend to be compacted into the foramen, which may cause apical blockage and interfere with the working length. The repeated penetration of the apical foramen with a file of adequate size during instrumentation prevents the accumulation of debris in this area leaving the foramen unblocked, i.e., patent. This concept has been defined as apical foramen patency. [9]

Therefore , establishing patency is leaving the apical foramen accessible, free from dentin chips, pulp fragments and other debris. [2]

Some authors have suggested that apical patency should be gained with an instrument that binds to the foramen. However, one of the arguments against this procedure is that a file that binds to the foramen will act like an embolus, increasing the possibility that debris are inadvertently extruded beyond the apex. [9] On the other hand, the use of a file that is not adjusted to the apical portion will offer a lesser risk of extrusion of debris or, at least, minimize its occurrence. Considering that the purpose of this procedure is to prevent the accumulation of dentin chips in the apical area, the use of an instrument of smaller size than the foramen will be effective with the advantage of offering a

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lesser risk of displacement of toxic products and dentin fragments from the root canal into the peri-apical space. [9]
While Buchanan [1], pointed out that apical canal blockage can be avoided during instrumentation using a patency file. He defines a patency file "as a small flexible K-file, which will passively move through the apical constrictor without widening it."
The apical limit of root canal instrumentation is still a very controversial topic in endodontics. The possibility of aggressions to apical and periapical tissues has supported the principle of the working length staying short of the radiographic apex. Different working lengths have been proposed, but the most widely accepted approach seems to be choosing a working length of 1 mm coronal to the root apex. [9]
Lambrianidis et al [5] evaluated the roles of the apical constriction and foramen and the use of a patency file on the apical extrusion of NaOCl and debris. The results indicated that there was significant difference in the amounts of extruded material before and after the enlargement of the apical constriction with greater extrusion when the constriction remained intact. Goldberg & Massone [2] assessed the transportation produced at the apical foramen when stainless steel or nickel titanium K-files #10, #15, #20, and #25 were used as a patency file. They showed that transportation of the apical foramen was detected in 18 of the 30 specimens analyzed. No statistical differences were observed when stainless steel or nickel-titanium K-files were used as a patency file. They suggested that if a patency file is used, one should use the smallest file size possible.
Tinaz et al [10] compared the amount of apical extrusion during manual instrumentation and engine-driven rotary instrumentation in teeth with disrupted apical constriction. They found that there was a tendency with both techniques to extrude apically more material as the diameter of the apical patency increased.
Leonardi et al [6] assessed the presence of apically extruded debris and evaluated the influence of canal curvature on the amount of debris produced by manual and mechanical techniques. They found no statistically significant difference between the manual and mechanical instrumentation, and no statistically significant differences were found between slight and moderate curvatures in terms of the amount of extruded debris.
Kustarci et al [3] compared the amount of debris and irrigant extruded apically, using manual technique and crown-down pressure less technique by K3, RaCe, and FlexMaster instruments. They found that all instrumentation techniques produced extruded debris and irrigant; however, the engine-driven nickel-titanium systems were associated with less apical extrusion and irrigant. This in vitro study tried to compare the apical foramen patency for two different instrumentation techniques (hand step-back & rotary crown-down).

METHODS

Thirty single-rooted human teeth were used in this study. Visual examination had been done to ensure the existence of a completely formed apex and the presence of straight roots & the teeth then were stored at room temperature in distilled water. The teeth were de-coronated at CEJ using carborandom disk in low speed hand piece. Size #15 K-file was extended just beyond the apical foramen to ensure the patency of the root canals before instrumentation, the same file was used for working length determination by subtracting 0.5 mm from this length.
The roots were mounted in holes cut through pieces of rubber dam covering plastic containers and these rubbers were secured in place with the aid of wires tightened around the plastic containers which with the roots in placed were weighed on an electronic balance to the third decimal digit.

The irrigation solution used was 5.25% NaOCl. The collection vials were hand-held vertically at all times. The roots were divided into two groups, the first 15 were instrumented using a step-back technique & other 15 instrumented using crown-down technique. The two instrumentation techniques were used twice; once without a patency file & the other with a patency file.

In the first group in which the step-back technique was used without the patency file, the canals enlarged till no. #35 file to the apical constriction and sizes #40, #45 & #50 were 1, 2 & 3 mm short of the working length respectively, after each instrument the canal was irrigated by 0.5 ml NaOCl irrigation solution with constant delivery every second instrument by means of a 5-ml plastic syringe with an attached 23-gauge endodontic needle except for last recapitulation step with the MAF # 35 in which 1.5 ml was delivered into the canal. The needle tip was loosely placed in the canal. In total, 5 ml of NaOCl per root were used. The collection vials (the bottles) were weighed after instrumentation, i.e. with the irrigating solution (the amount of NaOCl + debris that was extruded through the apical foramen into the collection vials) on an electronic balance to the third decimal digit.

The same roots were instrumented again in which a patency file was used. The roots were replaced on the washed and dried vials and new root canal instrumentation followed starting with a #50 file that was worked to 3 mm short of the original working length to create a ledge till #70 which considered as MAF and then continued with sizes #80, #90 & #100 files which were used coronally 4, 5 & 6 mm to the enlarged apical constriction respectively. They were then hand-held and the apical patency was done with a #25 file protruding apically 3 mm more than the new working length. After each instruments the canal was irrigated by 0.5 ml NaOCl irrigation solution with constant delivery every second instrument by means of a 5-ml plastic syringe with an attached 23-gauge endodontic needle except for the last step with the patency file in which 1.5 ml was delivered into the canal. The needle tip was loosely placed in the canal. In total, 5 ml of NaOCl per root were used. The collection vials (the bottles) were weighed after instrumentation, i.e. with the irrigating solution (the amount of NaOCl + debris that was extruded through the apical foramen into the collection vials) on an electronic balance to the third decimal digit.

The other 15 roots were instrumented using crown-down technique without a patency file, in which instrumentation started with S1 & S2, F2, F3 and F4 Protaper rotary system. Irrigation was identical to that used during step-back root canal preparation but 1ml of NaOCl was used after each instrument, in total, 5 ml of NaOCl per root were used. The collection vials (the bottles) were weighed after instrumentation, i.e. with the irrigating solution (the amount of NaOCl + debris that was extruded through the apical foramen into the collection vials) on an electronic balance to the third decimal digit. The same roots were instrumented again in which a patency file was used. The roots were replaced on the washed and dried vials and new root canal instrumentation followed starting with G.G drill that was worked to 3 mm short of the original working length to create a ledge, no. 6 G.G drill was used coronally ½ mm of the new working length, followed by no. 5 G.G drill inserted 2/3 of the new working length. Then F4 & F5 Protaper system was used to the new working length which is 3 mm shorter from the original working length. They were then hand-held and the apical patency was done with a #25 file protruding apically 3 mm more than the new working length. After each instruments the canal was irrigated by 1 ml NaOCl irrigation solution. In total, 5 ml of NaOCl per root were used. The

collection vials (the bottles) were weighed after instrumentation, i.e. with the irrigating solution (the amount of NaOCl + debris that was extruded through the apical foramen into the collection vials) on an electronic balance to the third decimal digit. The results for weight of extruded NaOCl-debris were subjected to statistical analysis using Student's t test

RESULTS

The descriptive statistics for the results had been shown in (Table 1).

By using t- test, there was non-significant difference between the Group A1 & A2 (step-back hand instrumentation without & with use of patency file) at p> 0.05, with in favor of without use of patency file group that showed the least debris extrusion mean value of 1.115 g. There was highly significant difference between the Group B1 & B2 (crown-down rotary instrumentation without & with use of patency file) at p< 0.01, with in favor of without use of patency file group that showed the least debris extrusion mean value of 0.704 g.

Table 1. Descriptive Statistics.

Groups	N	Minimum	Maximum	Mean	Std. Deviation
A1	15	.03	3.11	1.1151	1.08535
A2	15	.00	3.20	1.6311	1.43162
B1	15	.04	1.82	.7049	.49708
B2	15	.13	3.02	1.4856	1.11068

lesser debris extrusion than the hand step-back technique both with & without use of patency file, however, the difference was non-significant at p> 0.05.

For all the groups, the rotary crown-down technique showed lesser debris extrusion than the hand step-back technique, however, the difference was non-significant at p> 0.05.The use of patency file increase the extrusion than the other condition & the difference was highly significant at p< 0.01,(Tables 2,3) & (Figure1).

Table 2.Paired sample comparison.

Pairs	Groups	Mean	N	Std. Deviation	Std. Error Mean
I	A1	1.1151	15	1.08535	.28024
	A2	1.6311	15	1.43162	.36964
II	B1	.7049	15	.49708	.12834
	B2	1.4856	15	1.11068	.28678
III	A1	1.1151	15	1.08535	.28024
	B1	.7049	15	.49708	.12834
IV	A2	1.6311	15	1.43162	.36964
	B2	1.4856	15	1.11068	.28678
V	A1+A2	1.3731	30	1.27553	.23288
	B1+B2	1.0953	30	.93404	.17053
VI	A1+B1	.9100	30	.85526	.15615
	A2+B2	1.5583	30	1.26113	.23025

Table3. Paired sample t-test.

Pair of differences	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
A1-A2	-.51600	.99593	.25715	-1.06753	.03553	-2.007	14	.065
B1-B2	-.78067	.90051	.23251	-1.27935	-.28198	-3.358	14	.005
A1-B1	.41013	1.22661	.31671	-.26914	1.08941	1.295	14	.216
A2-B2	.14547	2.03578	.52564	-.98191	1.27284	.277	14	.786
A-B	.27780	1.65686	.30250	-.34088	.89648	.918	29	.366
1-2	-.64833	.94257	.17209	-1.00029	-.29637	-3.767	29	.001

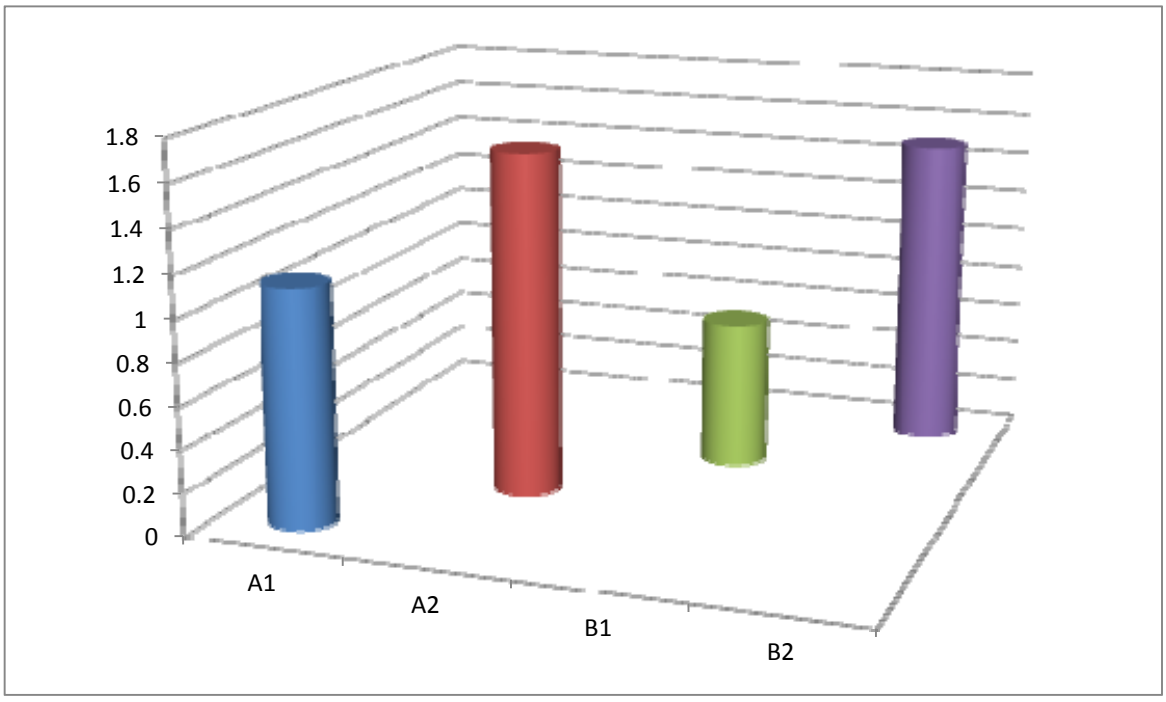

Figure 1. Bar chart illustrating the differences between the groups

DISCUSSION

Mid treatment flare-up is a common problem that practitioners encountered during root canal therapy. A major reason cited for such a distressing occurrence is the extrusion of debris present within and created during the instrumentation of the root canal system into the periradicular region, resulting in a persistent periapical inflammation. The results of apically extruded debris were found to be the preparation up to the apex, the diameter of the apical patency, the amount of irrigant used, formation of a dentin plug, the use of a step-back vs. crown-down technique, and the use of conventional hand filing vs. rotary motion, all have a correlation to the amount of extruded debris.

This study compared the hand step-back and rotary Protaper instrumentation

techniques in relation to extrusion of debris and irrigating solution with and without apical patency. In this study there was a highly significant difference between the group that patency file was used & the one that patency file was not used when hand step-back technique was used. Agreed with this Lambrianidis et al at 2001 in which they used step-back technique & NaOCl as irrigant, and found a significant difference in the amounts of extruded material before and after the enlargement of the apical constriction with greater extrusion when the constriction remained intact. While in case of rotary crown-down technique, there was a significant difference between the same types of groups although there was no significant difference between hand step-back & rotary crown-down techniques in relation to whether patency file was used or not.

Some authors prefer to use sterile water as irrigants instead of NaOCl which is used clinically because the latter may produce crystals that could result in an increase in weight. [6]

Tinaz et al [10] used NaOCl & revealed no significant difference between instrumentation with K-files and ProFile .04 taper files in relation to the amount of apical extrusion. There was a tendency with both techniques to extrude apically more material as the diameter of the apical patency increased. Logani and Shan [7] evaluated the amount of extruded debris using ProTaper hand, ProTaper rotary and ProFile, and the sterile water as an irrigant between instrumentation. They concluded that all rotary instruments tested produced apical extrusion of debris and that the ProTaper rotary extruded a significantly higher amount of debris than the Pro File.

Kuştarci et al [4] compared in vitro the amount of debris and irrigant extruded apically, using manual technique and crown-down pressure-less technique by K3, RaCe, and FlexMaster instruments. They used NaOCl as irrigants. They noted that all instrumentation techniques produced extruded debris and irrigant; however, the engine-driven nickel-titanium systems were associated with less apical extrusion and irrigant. Reddy & Hicks at 1998 used step-back instrumentation with K-files, balanced force with Flex-R files, Light-speed nickel titanium instruments, and .04 taper ProFile rotary nickel-titanium files, and found that all instrumentation techniques produced apically extruded debris, however, step-back instrumentation produced significantly more debris than the other methods. There was no difference between balanced force hand instrumentation and the two rotary nickel-titanium instrumentation methods. Hand or engine-driven instrumentation that uses rotation seems to reduce significantly the amount of debris extruded apically when compared with a push-pull technique.

The results of this in vitro study should not be directly extrapolated to clinical practice, since the presence of periapical and pulpal tissue may show resistance to apical extrusion of debris. Furthermore, measuring the amount of extruded debris in terms of weight is not adequate enough to make a speculation concerning a mid-treatment flare-up. There may be other factors such as extruded irrigant, intra-canal medication, virulence of bacteria and the host response that can trigger such a flare up. [7]

CONCLUSIONS

It was appeared that using of a patency file appears to be more important than the technique of instrumentation of the canals in relation to the extrusion of debris & irrigating solution.

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پوختە

توانای داخستنی لوتکەیی لە گەڵ دیاری کردنی کاریگەری دووان لەتەکنیکەکانی ئامادە کردن لەسەر پرکردنەوە و داخەری لوتکەیی.

پێشەکی:لەم توێژینەوەدا بە راوردکردنی توانای داخستنی لوتکەیی بۆ سێ تەکنیکی پرکردنەوە و دوو جۆر داخەر کرا، لە گەڵ دیاری کردنی کاریگەری دووان لەتەکنیکەکانی ئامادە کردن لەسەر پرکردنەوە و داخەری لوتکەیی.

سەد و بیست ددان کۆ کرانەوە ،پاشان بەشێوەیەکی هەرەمەکی دابەش کران بۆ دوو گرووپ ، 30 ڕەگ ئامادە کران بە بەکارهێنانی تەکنیکیی (step-back), 30 ڕەگەکەی تر ئامادە کران بە بەکار هێنانی تەکنیکی(step down) , بۆ لابردنی هەر کۆمەڵەیەك لە ددانە ئامادەکراوەکان بەشێوەیەکی هەرەمەکی دابەشکران بەسەر سێ کۆمەڵەی پرکردنەوە.

ئەنجام :لەمەوە بۆمان دەردەکەوێت : کە بەکارهێنانی پەستێتۆرانی شاقوولّی گەرم وەك تەکنیکی پرکردنەوە بە پەیوەست بوون لەگەڵ داخەری هەڵدەستێت .

دەرئەنجام:بەئەنجام دانی پرکردنەوەی ڕەگەکان بەکەمترین بڕی لێچوون وتەکنیکیstep-back باشترین شێوە دەدات بە ڕەگەکان لەرووی داخستنی لووتکەیی.

الخلاصة

تاثير أثير الإنفتاح القممي على نتوءالمجاور للقمة أثناء تقنيات مختلفة

مقدمة:هذه الدراسة اجريت على 30 سن لقناة واحدة وقطعت ووضعت في فتحة لمحتوى بلاستيكي وقسمت الاسنان الى مجموعتين: المجموعة الاولى تحضر بطريقة التقليل الى اعلى والمجموعة الثانية بطريقة التقليل الى اسفل وكل مجموعة استخدمت معها المبرد الفاتح او لم يستخدم.

النتائج :اظهرت انه لا يوجد فرق معنوي بين المجموعتين ويوجد فرق معنوي عند استخدام المبرد الفاتح, كما اظهرت النتائج ان طريقة التقليل الى اسفل تفرز اقل من البرادة عن الطريقة الاخرى.

استنتاج: يوجد ارتباط قوي بين استخدام المبرد الفاتح والبرادة الناتجة.

ANTI-CCP AS A NEW SEROLOGIC DIAGNOSTIC MARKER FOR THE DIAGNOSIS OF RHEUMATOID ARTHRITIS PATIENTS

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ABSTRACT

Background and objective: Rheumatoid arthritis (RA) is a systemic autoimmune disease of unknown etiology with chronic joint inflammation. Anti-cyclic citrullinated peptide (anti-CCP)antibody testing is particularly useful in the diagnosis of RA, with high specificity. These antibodies also identify a subset of patients who are likely to have substantial ongoing disease activity, more damage, and who will probably benefit most from early aggressive treatment. This study was conducted to assess the diagnostic value of anti-CCP RA patients.
Methods: This study was carried out on 90 RA patients 17(18.9%) males and 73(81.1%) females who admitted Rizgari Teaching Hospital and 30 healthy controls (HC) in a period between November 2008 and November 2009. Parameters like RF-latex, erythrocyte sedimentation rate(ESR), C-reactive protein (CRP)tests were performed on 90 RA patients and 30 HC. Study groups were tested for enzyme linked immuno-sorbent assay (ELISA) anti-CCP, RF-IgM, RF-IgG and RF-IgA.
Results: Out of 90 RA patients assessed, 60(66.7%) were RF-latex test positive. Regarding RA serological markers, the best specificity and sensitivity recorded by anti-CCP antibodies were (96.2%) and (73.7%). Anti-CCP test showed significant and highly significant correlation with RF-latex and RF-isotypes respectively. Moreover, a significant difference ($P<0.05$) was observed in mean serum concentration between anti-CCP+ve and anti-CCP-ve patients concerning RF-(latex and IgA) and $P<0.01$ was observed regarding RF-(IgM and IgG).
Conclusion: Anti-CCP mostly considered as diagnostic disease specific RA marker.

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Key words: Rheumatoid arthritis; Anti-cyclic citrrulinated peptides; Rheumatoid factor

Rheumatoid arthritis is a chronic progressive autoimmune disease with varying systemic features that affects multiple tissues but principally attacking the joints to produce a non suppurtive proliferative synovitis that frequently progresses to destroy articular cartilage and the underlying bone with resulting disabling arthritis[1].

The disease is much more common in women than men with a ratio 3:1 while the most common age of onset is between the ages of 30 and 50 years, but over the age of 60 years the disease occurs with equal incidence in men and women[2]. Because the diagnosis of RA is primarily based on clinical symptoms, so it is often difficult to diagnose RA in very early stages of the disease [3]. Furthermore, RF has a limited specificity for RA, therefore other more specific antibodies have been sought[4]. Most promising for the future appears to be the growing family of antigens containing one or more citrulline residues. Some of these antigens have recently been applied in simple and easy to use as ELISA tests, and have been shown to be very specific for RA, to be able to diagnose RA in a very early phase of the disease, and to predict erosive disease[3].

The serological parameter that meets these requirements for a good and useful marker for RA is the anti-citrullinated peptide antibody[5]. The sensitivity of this antibody is comparable to that of the RF

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(approximately 80%), but its specificity is much higher, about 98%. Several assays have been developed to detect this class of auto-antibodies, which are termed anti-cyclic citrrulinated peptides (CCP) antibodies because the most sensitive test is based upon CCP5.

Indeed it has been found that IgM-RF is often found in the same patients, but with much lower specificity for RA. Additionally, Anti-CCP antibodies may pre-date arthritis by several years[6].

Although citrulline is a common metabolite present throughout the human body, it is a non-standard amino acid, which means that it can not be incorporated into peptide during protein synthesis[7].

Citrulline-containing peptide can only be generated through post-translational modification of arginine residues, a reaction that is catalysed by peptidylarginine deiminase (PAD) enzymes[7].

It is possible that environmental factors may locally induce abnormal cell death or disturb the clearance of apoptotic cells. Subsequently, citrullinated protein fragments may be presented to the immune system.

A primary and specific immune response will then develop. The resulting auto-antibodies will recognize epitopes on apoptotic cells that express auto-antigenic molecules at the cell surface, and such opsonized apoptotic cells will generate further pro-inflammatory responses [8].

METHODS

This case-control study comprised 90 Iraqi patients, 17(18.9%) males and 73(81.1%) females were diagnosed on the basis of clinical and laboratory ground to be with RA and satisfying at least 4 of 7 of the revised criteria for RA diagnosis which were defined by the American College of Rheumatology 19879. They were attending or admitted to Rizgari Teaching Hospital between November 2008 and November 2009. The mean age of total 90 patients was 44.29±1.24 and their ages ranged from 19 to 72 years with disease duration ranged between 2 months to 30 years. Also 30 healthy individuals as healthy control had been chosen from hospital medical staff. Exactly (61) from (90) RA patients and (27) from (30) HC subjects had been randomly selected to be tested for different immunological tests, which include the following tests: -

Qualitative and semi-quantitative measurement of rheumatoid factor (RF) and C-reactive protein (CRP) by latex test.

Quantitative measurement of anti-cyclic citrullinated peptide (CCP) antibodies and RF- immunoglobulin (M, G and A) isotypes by ELISA.Erythrocyte sedimentation rate (ESR).

RESULTS

The females at a higher risk for RA with sex ratio (4:1). RF+ve patients were predominance 60(66.7%) vs. 30(33.3%) in RF-ve with (P<0.05)(Table1)

Table 1.Gender frequency of RF-latex test in RA patients

	Gender	RA patients No.= 90 Female No.=73		Male No.=17		Total No.=90		P value Chi–square
		No.	%	No.	%	No.	%	*P<0.05: Significant
Latex test	Seropositive	45	61.6	15	88.2	60	66.7	
	Seronegative	28	38.4	2	11.8	30	33.3	P< 0.05*
	Total	73	100	17	100	90	100	

The demographic comparison between studied groups showed that the mean concentration of RF-latex, ESR and CRP in RA patients were higher compared to HC subjects (Table 2).

Table 2. Demographic characters of studied groups

Character	Patient No.90		HC No.30	
	Mean ±SE	Range	Mean ±SE	Range
Age in years*				
Male	45.35±3.38	19-70	41.33±3.59	28-51
Female	44.32±1.33	17-72	42.46±1.87	27-55
RF-Latex**	403.03±87.18	0-4096	2.67±1.35	0-32
ESR mm/1 hr***	45.98± 2.74	5-113	10.7±1.25	2-28
CRP****	54.00±7.87	0-384	0.00±0.00	0-0

HC: Healthy control.
**Mean ages of total 90 patients=44.29±1.24 and of total 30 HC=42.23±1.64.*
***Normal level of RF= <8 IU/ml.*
**** Normal value of ESR (adult) = <17 mm/hr (Mal) and <25 mm/hr (Female).*
***** Normal level of CRP= <6 mg/I.*

(Table 3) revealed the specificity, sensitivity of different RA immunological tests. The best specificity and sensitivity recorded by using anti-CCP antibodies were (96.2%) and (73.7%).

Table 3. Sensitivity and specificity of Immunoparameters in RA patients

Immunological Parameters	Sensitivity %	Specificity %
ELISA anti-CCP	73.7	96.2
ELISA IgM–RF	65.5	88.8
ELISA IgG –RF	70.5	85.2
ELISA IgA–RF	42.6	92.5
RF–Latex	63.9	85.1

The mean serum concentration of anti-CCP positive (anti-CCP+ve) RA patients group differed if compared with both groups anti-CCP negative (anti-CCP-ve) RA patients and healthy subjects (P<0.01) (Table 4).

The presence or absence of RF-(IgM and IgG) respectively in anti-CCP+ve patients differed with highly significant in comparison to their presence or absence in anti-CCP-ve patients (P<0.01). While, (P>0.05) for IgA-RF+ve or IgA-RF-ve in anti-CCP+ve in comparison to IgA-RF positivity and its negativity in anti-CCP-ve patients (Table 5).

Regarding correlation between Anti-CCP, IgM-RF and RF-latex with other RF-isotypes in RA patients, (Table 6) demonstrates a significant correlation of anti-CCP with RF-latex (P<0.05), but it's correlation with all three RF-isotypes was highly significant (P<0.01).

Comparisons mean serum concentration of different RA markers in anti- CCP+ve and -ve groups revealed significant result (Table 7).

A CRP differ significantly in anti-CCP+ve and -ve, but morning stiffness and ESR tests were (P<0.01) and (P>0.05) for both sexes in RA patients (Table 8).

Table 4. Serum anti-CCP antibodies (U/ml) between studied groups.

	Study Groups		No.	Serum Anti-CCP Mean ±SE	P value (F-test)
Serum anti-CCP	Positive RA patients		45	1743.98±141.35	
	Negative RA patients		16	3.06 ± 1.43	P< 0.01
	HC		27	3.11± 1.11	
	Positive RA patients vs HC	T test		P< 0.01	
	Negative RA patients vs HC			NS	
	Positive RA patients vs negative RA patients			P< 0.01	

**P<0.01: Highly significant; NS: Non significant (P> 0.05); HC: Healthy control*

Table 5. Frequency of RF-isotypes in anti-CCP^{+ve} and anti CCP^{-ve} patients.

RA Immunological tests		Anti-CCP ELISA test No. 61				Pearson Chi-Square	P value
		Positive No.=45	%	Negative No.=16	%		
IgM-RF	+	34	75.6	6	37.5	7.572	*P<0.01
	-	11	24.4	10	62.5		
IgG-RF	+	36	80	7	43.8	7.457	P<0.01
	-	9	20	9	56.3		
IgA-RF	+	21	46.7	5	31.3	1.147	NS
	-	24	53.3	11	68.8		
All three RF-isotopes	+	18	40	4	25	5.596	**P<0.05
	-	4	8.9	6	37.5		
At least one RF isotope	+	9	20	6	37.5	9.957	P<0.01
	-	14	31.1	0	0		
At least two RF	+	14	31.1	0	0	9.957	P<0.01
	-	9	20	6	37.5		
One or more RF-isotypes	+	41	91.1	10	62.5	16.944	P<0.01
	-	4	8.9	12	75		

**P<0.01: Highly significant; **P< 0.05: significant; NS: Non significant (P> 0.05).*

Table 6. Correlation of anti-CCP, IgM-RF and RF- latex with RF-isotopes.

Parameters	Anti-CCP	RF-latex	IgM-RF
RF-latex			
Pearson correlation	0.294	–	–
Sig (2-tailed)	P < 0.05*		
IgG-RF			
Pearson correlation	0.352	0.235	0.282
Sig (2-tailed)	P < 0.01**	NS	P<0.05
IgM-RF			
Pearson correlation	0.512	0.731	–
Sig (2-tailed)	P < 0.01	P < 0.01	
IgA-RF			
Pearson correlation	0.395	0.603	0.659
Sig (2-tailed)	P < 0.01	P < 0.01	P < 0.01

* *Correlation is significant at (0.05) level (2-tailed).*
** *Correlation ishighly significant at (0.01) levels (2-tailed); NS: Non significant.*

Table 7. Comparison in means of RA markers in anti- CCP$^{+ve \text{ and } -ve}$ groups.

Immunological Parameter	Anti-CCP^{ve+} No.=45 Mean ±SE	Anti-CCP^{ve-} No. =16 Mean ±SE	P value T-test
RF-latex	573.51±139.15	128.0± 28.0	P<0.05
IgG-RF	183.24±24.5	54.38±23.94	P<0.01
IgM-RF	122.00±15.21	29.97±13.15	P<0.01
IgA-RF	92.58±20.25	32.25±14.38	P<0.05

Table 8. Frequency of different variables in anti-CCP$^{+ve \text{ and } -ve}$ patients.

Variable	Anti-CCP/ No.= 61 +ve No.= 45 No (%)	-ve No.=16 No (%)	Pearson's Chi square	P value
`CRP				
>6 mg/l	42(93.3)	12(75)	3.905	P< 0.05*
<6mg/l	3 (6.7)	4(25)		
ESR				
M >17mm/hr	8(17.8)	1(6.3)	0.207	NS
M <17mm/hr	4(8.9)	1(6.3)		
F >25mm/hr	30(66.7)	12(75) 2(12.5)	0.279	NS
F<25mm/hr	3(6.7)			
Morning >1 hr	37(82.2)	3(18.8)	21.064	P<0.01**
Stiffness <1 h r	8(17.8)	13(81.3)		

**P< 0.05: Significant; **P<0.01: Highly significant; NS: Non significant (P> 0.05).*
M= Male, F= Female.

Morning stiffness had a highly significant correlation with anti-CCP but inverse result with CRP and ESR P>0.05(Table 9).

Table 9.Correlation between serological tests with disease activity markers.

Inflammatory Parameter	Anti-CCP	IgM-RF	RF- Latex
CRP			
Pearson correlation	**0.197**	**0.532**	**0.637**
Sig. (2-tailed)	**NS**	**P< 0.01**	**P< 0.01***
ESR			
Pearson correlation	**0.136**	**0.290**	**0.235**
Sig. (2-tailed)	**NS**	**P<0.05****	**NS**
Morning stiffness			
Pearson chi square	**21.06**	**14.746**	**27.983**
Sig. (2-tailed)	**P< 0.01**	**P< 0.01**	**P< 0.01**

The P>0.05 of both CRP (69.78±12.95) and ESR (55.13±4.41) in anti-CCP+ve patients compared with mean serum concentration (36.75±12.94) and (41.31±5.49) of CRP and ESR respectively, in anti-CCP-ve patients (P>0.05), (Table 10).

Table 10. Comparison activity markers in anti-CCP$^{+ve\ and\ -ve}$ patients.

Markers	anti-CCP^{+ve} /No.45 Mean ±SE.	anti-CCP^{-ve} /No.16 Mean ±SE.	P value T-test
CRP	**69.78±12.95**	**36.75±12.94**	**NS**
ESR	**55.13±4.41**	**41.31±5.49**	**NS**

NS: non significant (P>0.05)

(Table 11) demonstrates that P>0.05 of patients` age and history of RA in their family with each of anti-CCP and both IgM-RF and -RF latex, while gender as a risk factor showed no significant association with anti-CCP (p >0.05), but correlated significantly with both RF-latex and IgM-RF (p<0.05)

Table 11. Correlation of risk factors with RA markers in RA patients

Risk factors	Anti-CCP	IgM-RF	RF- Latex
Age			
Pearson correlation	**0.242**	**0.144**	**0.003**
Sig. (2- tailed)	**NS**	**NS**	**NS**
Gender			
Pearson chi square	**1.34**	**5.992**	**3.738**
Sig. (2- tailed)	**NS**	**P<0.05**	**P<0.05**
Family history			
Pearson chi square	**1.554**	**0.099**	**0.007**
Sig. (2- tailed)	**NS**	**NS**	**NS**

P< 0.05: Significant; NS: Non significant (p>0.05).*

DISCUSSION

It is generally accepted that the distribution of RA is usually predominant in women. Current data verified this concept as 73(81.1%) of patients were female and 17(18.9%) were male, P<0.01 with female to male ratio (4:1). Similar results were recorded in Iraq/ Baghdad city (4.5:1) and in Hawler city (5:1) [10,11] respectively. Indeed, out of 90 patients with RA 60(66.7%) were seropositive and 30(33.3%) were seronegative with (P<0.05), (Table 1). Hormonal factors like estrogen and progesterone could potentially explain some of the gender effect. Estrogen might have detrimental effects through its ability to decrease apoptosis of B cells, potentially permitting the selection of auto-reactive clones. Hormones also have a complex influence on the balance of T-cell subsets with distinct cytokine profiles[12]. In the present study the mean age of total 90 patients was 44.29±1.24, (Table 2). These results somewhat agree with previous studies done in Iraq [10-13], 42±11.3, 44.8±10.2 and 43.97 respectively.

Elevation of ESR and the CRP are frequent in RA. In aggressive or explosive disease, ESR may even approach 100 mm/h but CRP is considered by many rheumatologists to be a more sensitive indicator of inflammation and might be increased in circumstances in which the ESR is either normal or minimally elevated[14] (Table 2).

The best sensitivity in the present study was obtained by anti-CCP antibodies (73.7%) (Table 3) this is nearly agreed with results of other studies which are 70%, 72.3% and 72.8%14 and [16]. This level of sensitivity for anti-CCP antibodies test implies that the test can be successfully applied for the majority of RA patients. However, the results of several experiments indicate that the antibody response to citrullinated epitopes is strongly polyclonal[17]. By increasing the number of citrullinated epitopes that are presented in the anti-CCP test, it should be possible to increase the sensitivity of the assay [5]. RF-IgM in our result showed moderate specificity 88.8% which was not high as that found for anti-CCP test. The result of this study is consistent with percentage of other studies 84.6% and 86.4%[1,18], but not supported result of other studies 100%, 82.1% and 69%13; [19,20]. The variability in the sensitivity and specificity among different studies may relate to slightly different cut-off points for positivity, differences in disease duration, severity and other clinical characteristics of there groups being tested. It can also explain by differences in patients' selection and study designs [21].

Current study revealed highly P<0.01 of anti-CCP concentration in anti-CCP+ve RA patients when compared with anti-CCP-ve patients and HC, (Table 4). Currents results agree with results that obtained by Skaik, [20]. Although only a few studies have analyzed the relationship between anti-CCP antibody concentration and the patient's progress, antibody concentrations are probably important for prognostic and therapeutic reasons, because high anti-CCP concentrations on diagnosis have been shown to predict a more severe course of the disease [22].

According to the results of present study, patients who were anti-CCP+ve found to show higher frequency for RF-isotypes than those with anti-CCP-ve except RF-IgA (Table 5). Although the presence of these isotopyes in SLE indicated that RF is not specific for RA, but suggested more central role for RF in RA disorder because RF-seropositive RA patients had significantly greater concentrations of all classes of RF compared to RF seropositive SLE[23]. Current study revealed a significant correlation between anti-CCP and RF-isotypes (P<0.01) (Table 6). The same significant association results between anti-CCP, and RF isotypes present was found with Bizzaro et al [24] who showed such association between anti-CCP and all RF-isotypes.

This provided an additional diagnostic value of anti-CCP since it confirmed the presence of RA in those patients in whom the presence of the disease was uncertain or they were suspected to have RA, once again because of high anti-CCP specificity for this disease. Evidence has suggested that RF and anti-CCP antibodies are two separate auto-antibodies. Data from De Rycke et al, [25] indicate that the RF and anti-CCP antibodies may provide different and eventually, complementary biological information on the disease process in RA. The anti-CCP could act as a disease specific marker for RA, whereas RF titers could be related to disease activity [25]. In contrast to anti-CCP, RF-IgM showed a significant relation with ESR ($P<0.05$) and highly significant relation was found between both RF-latex and RF-IgM with CRP ($P<0.01$) (Table 6). Also, (Table 7) demonstrated no significant difference between anti-CCP+ve and anti-CCP-ve in CRP and ESR ($P>0.05$). The same result was obtained by Lee et al, [26]. Numerous considerations could explain the lack of correlation between anti-CCP titer and other disease parameters like ESR or CRP or with age (Table 8) including auto-antibody isotype profile, autoantibody glycosylation state [27] or the subset of anti-citrulline autoantibodies captured in the commercial anti-CCP ELISA assays. This observation underscores the complexity of pathogenic processes in RA and the continued need for research into the role of auto-antibodies in this disease [26].

Interestingly, ESR test does not agree with the other disease activity markers that measured in current study such as CRP, morning stiffness that result of each one supported result of the other one as all of these tests showed significant difference in comparison to their presence or absence in positive and negative results for anti-CCP, (Table 8).

Numerous considerations could explain the lack of correlation between anti-CCP titer and other disease parameters like ESR or CRP or with age (Table 9) including auto-antibody isotype profile, autoantibody glycosylation state [27] or the subset of anti-citrulline autoantibodies captured in the commercial anti-CCP ELISA assays. This observation underscores the complexity of pathogenic processes in RA and the continued need for research into the role of auto-antibodies in this disease [26] .Also, (Table 10) demonstrated no significant difference between anti-CCP+ve and anti-CCP-ve in CRP and ESR ($P>0.05$). The same results were obtained by Lee et al, [26].Anti-CCP showed no correlation with age, gender and family history of patients group. Both RF-latex and RF-IgM showed same results except that for gender, (Table 11). Such results reported by many [15,20,26] . In addition to what mentioned above result of current study regarding age could be explain that the disease can be occur at any age because no age is protect from the disease , while hormonal variation may affect on auto-antibodies production especially in the presence of certain shared epitope. Further genetic studies required to found out any genetic risk factor in patients with family history.

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پوختە

دژەتەنی CCP وەکو نیشانەیەکی سیرەمی دەستنیشانکردنی نوێ بۆ دەستنیشانکردنی نەخۆشیەکانی جومگەی رۆماتیزمی دڕێژخایەن

ئامانج: نەخۆشی هەوکردنی جومگەی رۆماتیزمی دڕێژخایەن لە نەخۆشیانەیە کە توشی بەرگری خود دەبنەوە لە هەموو کۆئیندامەکانی لەش. وە نەخۆشیەکەکە هۆکاری دروست بوونی نادیارە لەگەل زۆربەی هەوکردنەکانی جومگەی رۆماتیزمی دڕێژخایەن. دژەتەنی Anti-CCP بەسوودە بۆ دیاریکردنی نەخۆشی هەوکردنی جومگەی رۆماتیزمی دڕێژخایەن، وە تایبەتمەندییەکی بەرزی هەیە. ئەم دژە تەنانە هەروەها بەکاردێت بۆ دیاریکردنی کۆمەلەیەکی زۆری تری نەخۆشی کە گریمانەی چالاکی نەخۆشیەکە دیاری دەکەن وە بەکاردێت بۆ دیاریکردنی زەرەمەندییەکانی نەخۆشیەکە، و دیاریکردنی ئەوانەی سوود لە چارەسەری زووی بەهێز وەردەگرن. ئەم توێژینەوە ئەنجام دراوە بۆ دیاریکردنی بەهای دەستنشان کردنیAnti-CCP بۆ نەخۆشی هەوکردنی جومگەی رۆماتیزمی دڕێژخایەن.

ڕێگا شەکولینی: ئەم توێژینەوە ئەنجامدرا لەسەر (90) نەخۆشی هەوکردنی جومگەی رۆماتیزمی دڕێژخایەن کە 17 (18.9٪) من نێر و 73 (81.1٪) مێ بوون ئەوانەی کە سەردانی نەخۆشخانەی رزگاری یان کردبوو لەگەل ئەوانەش 30 کەسی ساغ یش وەرگیراون لە نێوان ماوەی مانگی تشرینی دووەمی 2008 تا مانگی تشرینی دووەمی 2009. ئەو پارامیتەرانەی وەرگیراون وەک: لاتێکس RF، ڕێژەی نیشتنی خرۆکە سوورەکان .E.S.R و CRP. توێژینەوەی تاقیگەیی لەسەر هەر 90 نەخۆش و 30 ساغەکە ئەنجامدرا بە ڕێگەی (ELISA) بۆ دیاریکردنی دژەتەنی CCP و RF-IgM, RF-IgG and RF-IgA.

ئەنجام: لە (90) نەخۆشی جومگەی رۆماتیزمی دڕێژخایەن 60 (66.7٪) لەوانە پشکنینی لاتێکس پۆزەتیڤ بوو، وەسەبارەت بە نیشانەی سیرەمی نەخۆشی جومگەی رۆماتیزمی دڕێژخایەن بۆمان دەرکەوت کە تایبەتمەندی و هەستیاری دژەتەنی CCP (96.2٪و73.7) ٪.و دژەتەنەکە دەریخست کە پەیوەندیەکی گەورە و گرنگ هەیە لەگەل پشکنینی لاتێکس و RF isotypes یەک لەدوای یەک.

سەرەڕای ئەمانە بینرا کە جیاوازییەکی مەعنەوی هەیە لە نرخی (P <0.05) لە چری سیرەم بۆ دژەتەنی CCP پۆزەتیڤ لە نەخۆش لەگەل نێگەتیڤ بە پەیوەندی لەگەل RF-(latex and IgA) وە تێبینی کرا کە نرخی P <0.01 سەبارەت بە RF-(IgM and IgG).

دەرەنجام : دژە تەنی CCP دادەنرێت بە نیشانەیەکی تایبەتی دەستنیشانکردنی نەخۆسی جومگەی رۆماتیزمی دڕێژخایەن.

الخلاصة

مضادة CCP كعلامة مصل تشخيص جديدة لتشخيص مرضى المفاصل الرثياني

الخلفية والهدف: التهاب المفاصل الرثياني هو من الامراض المناعة الذاتية التي تصيب انظمة الجسم، وهو مرض غير معروف السبب مع التهاب المفاصل المزمنة. اختبار Anti-CCP مفيد بشكل خاص في تخيص التهاب المفاصل الرثياني، مع خصوصية عالية. هذه الأجسام المضادة أيضا تحدد مجموعة فرعية من المرضى الذين من المحتمل أن يكون نشاط المرض كبيرة لديهم. ويحدد مزيد من الضرر للمرض، ويحدد الذين سيستفيدون من التكبير بالعلاج القوي. وقد أجريت هذه الدراسة لتقييم القيمة التشخيصية لمضادة CCP لمرضى التهاب المفاصل الرثياني.

طرق البحث:أجريت هذه الدراسة على (90) مرضى التهاب المفاصل الرثياني 17 (18.9٪) من الذكور و 73 (81.1٪) من الإناث، من المرضى الذين دخلوا مستشفى رزكارى التعليمي وتناول البحث ايضاً 30 شخصاً صحيحاً بين فترة تشرين الثاني 2008 حتى تشرين الثاني 2009. وكانت المعلمات مثل اللاتكس– RF، معدل ترسيب كريات الدم الحمراء .E.S.R ، CRP، اجريت على ال 90 المريض وال30 الصحيح. تم اختبار مجموعات الدراسة لانزيم المناعي المرتبط مقايسة المواد الماصة (ELISA)المضادة للCCP،-RF .IgM, RF-IgG and RF-IgA.

النتائج: من ال(90) مريضاً من مرضى المفاصل الرثياني كان 60 (66.7٪) منهم اختبار اللاتكس موجب، وفيما يتعلف بالعلامات المصلية لمرضى المفاصل الرثياني، تبين أن أفضل خصوصية وحساسية التي سجلتها anti-CCP 96.2)٪ (و73.7) ٪ .(وأظهرت المضادة للاختبار CCPارتباط كبير وهام للغاية مع -RFاللاتكس و -RF isotypes على التوالي.

وعلاوة على ذلك، لوحظ وجود فروق معنوية ($P < 0.05$) في تركيز المصل بين المضادة لل CCP الايجابي للمرضى وبين المضادة السلبية بالعلاقة مع RF-(latex and IgA) ولوحظ $P < 0.01$ بشأن .RF-(IgM and IgG).

الاستنتاج: تعتبر مضادة CCP علامة خاصة في تشخيص مرض المفاصل الرثياني.

IMMUNOREGULATORY EFFECT OF LACTOBACILLI IN MACROPHAGES CELL LINE J744.1

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ABSTRACT

Ten Lactobacilli which showed high antagonistic activity against oral pathogen were selected among 111 lactobacillus strains and their immunomodulating effect was studied in both naïve and lipopolysaccharied induced murine macrophage cell line J774.1. Lactobacilli showed significant differences in the profile of released cytokines among the strains. All Lactobacillus strains exhibited TNF-α/ IL-10 ratio above 24:1, while one strain Lactobacillus acidophilus (K44/3) exhibited high ratio which was 110:1 indicating that this strain is the strongest for stimulating of pro-inflammatory cytokines.

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Key words: Lactobacilli, immunoregulation, macrophages

The genus Lactobacilli is a heterogeneous group of microorganisms comprising more than 70 species and subspecies with a broad ecological distribution [1]. Lactobacilli are one of the main constituents of the normal indigenous flora of humans, especially in the oral cavity, the female genital tract and the digestive tract, where they contribute to health [2,3]. Lactobacilli have long been used in the preparation of the fermented foods, in which form also they contribute to health [4]. Evidence from clinical and animal studies has supported the idea that Lactobacilli, especially some selected strains, can modify immune responses of the host [5]. However, the mechanisms by which Lactobacilli alter the immunological responses are unclear.

Macrophages are tissue – based phagocytes derived from monocytes. They participate in both innate and adaptive immune responses [6,7]. Macrphages are activated by microbial metabolites such as Lipopolysaccharide (LPS), molecules such as CD40 ligands and by T cell cytokines such as interferon –γ (IFN- γ). Activated macrophages phagocytose microorganisms; secrete proinflammatory cytokines and present antigens to helper T cells. Many of these activities are mediated through the release of different cytokines. Therefore, a change in profile of macrophages –derived cytokines can skew toward either humoral (Th1 cells activation) and cell mediated immune responses (Th2 cells activation). Recently researchers found that Bifidobacteria stimulate the production of macrophages-derived cytokines in a strain dependent manner [8].

The purpose of this paper was to study the ability of Lactobacilli to induce cytokines secretion by macrophages cell line J774.1.

METHODS

Bacterial Strains

Ten Lactobacillus strains were selected, Lactobacillus rhamnosus (31), Lactobacillus fermentum (19), Lactobacillus rhamnosus (7/1), Lactobacillus rhamnosus (37/1), Lactobacillus rhamnosus (38/1), Lactobacillus plantarum (K5), Lactobacillus acidophilus (K44/1), Lactobacillus rhamnosus (GG),

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Lactobacillus rhamnosus (K59/1) and Lactobacillus casei strain shirota as shown in (Table 1). These strains were chosen from 111 Lactobacillus strains which showed high antagonistic activity.

Table1. Lactobacillus strains and their origin.

Strain No.	Bacteria	Origin
31	Lactobacillus rhamnosus	Oral cavity
19	Lactobacillus rhamnosus	Oral cavity
7/1	Lactobacillus fermentum	Oral cavity
37/1	Lactobacillus rhamnosus	Human intestine
38/1	Lactobacillus rhamnosus	Human intestine
K5	Lactobacillus fermentum	Oral cavity
K44/3	Lactobacillus acidophilus	Oral cavity
K59/1	Lactobacillus rhamnosus	Oral cavity
GG	Lactobacillus rhamnosus	Human intestine
Shirota	Lactobacillus casei	Dairy food

The strains were prepared according to the method described by Morita et al. [9]. Briefly, Lactobacillus strains were cultured first in MRS (de Man, Rogosa and Sharpe) broth (Oxoid) for 18 h at 37 ºC two or three times. After incubation, the bacteria were collected by centrifugation and washed three times with phosphate-buffered saline (pH 7.1). The bacteria then suspended in RPMI (Roswell Park Memorial Institute) 1640 medium at 108 CFU/ml and heat inactivated at 70 ºC for 30 min.

Murine macrophage cell line J774.1

Macrophages were cultured in 24- well flat bottom cell culture plates at 5x 105 / well in RPMI 1640 medium (JR Scientific Inc., Woodland, CA, USA) supplemented with 5% FCS, at 37 ºC in an atmosphere of 5% CO2. Lipopolysaccharide (LPS) (Sigma-Aldrich, Germany) at 100 ng /ml was used for induction of cells. Cells were activated with tested bacteria at ratio of 200: 1 of bacteria: macrophages. After 24 h culture supernatants were collected and frozen until used.

Cytokine determination

Cytokine concentrations (IL-6), (IL- 10), (IL- 12p) and (TNF-α) were measured by 96-well plate reader (Power Wavex, Bio-Tek Instrument, Inc., USA) using capture ELISA as described by Marcinkiewicz et al.(10).

RESULTS

Murine macrophages produce small amounts of TNF-α and undetectable amounts of IL-6, IL-10 and IL-12P40. However, incubation of these macrophages with heat-killed Lactobacilli resulted in a massive release of a variety of pro-and anti-inflammatory cytokines such as IL-12 and Il-10.

All tested bacteria induced substantial secretions of TNF-α, IL-6, IL-10 and IL-12p40, showing significant differences in the profile of released cytokines among the strains. Among tested Lactobacillus strains, two strains (7/1, K5) induced significant levels ($p<0.05$) of TNF- α, IL-

6, IL-10 and IL-12p40 in both naïve macrophages and macrophages induced by lipopolysaccharide (LPS), while Lactobacillus strain (K44/3) induced significant level ($p<0.05$) of TNF- α in both naïve macrophages and macrophages induced by lipopolysaccharide (LPS) as shown in (Figures. 1 and 2).

All Lactobacillus strains exhibited TNF-α/ IL-10 ration above 24 pg/ml:1 pg/ml, while one strain Lactobacillus acidophilus (K44/3) exhibited high ratio which was 110 pg/ml:1 pg/ml indicating that this strains is the strongest for stimulating of pro-inflammatory cytokines

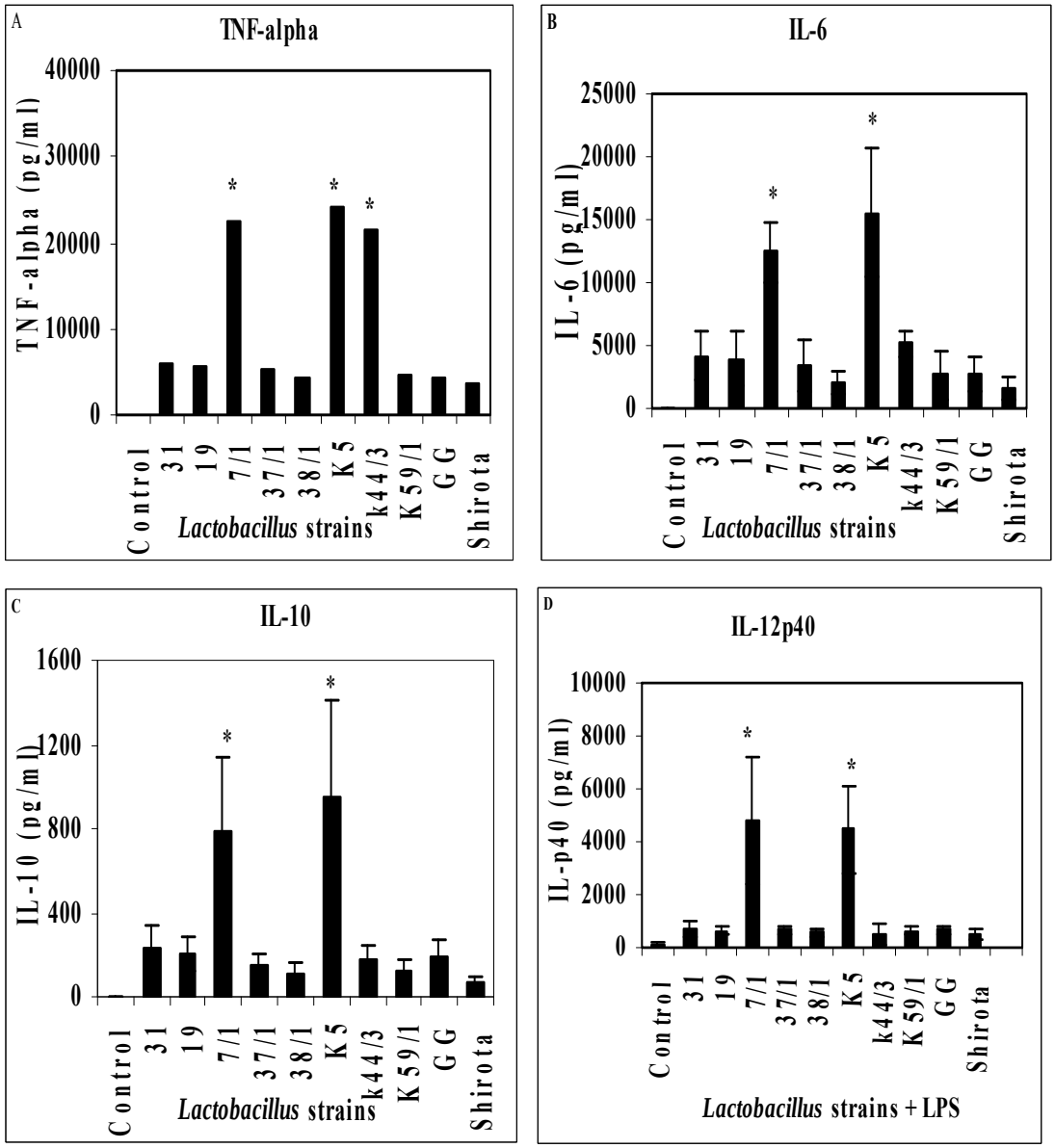

Figure 1. Cytokine induction by heat-killed Lactobacilli.Cytokines TNF-α (A),IL-6, (B),IL-10 (C)and(D) IL-12p40 were analyzed by commercial cytokine ELISA kits in supernatants collected from 24 h cultures of murine macrophages cell line J774.1(5x105 cell/well) incubated with different strains of heat-killed Lactobacilli (1X107CFU/ml). Data represent the average of three independent experiments and DS (error bar) of each cytokine in pg/ml. Statistical significance of the differences in the level of cytokines secretion induced by Lactobacilli was determined by analysis of variance (ANOVA) using Friedman test (p< 0.00091)

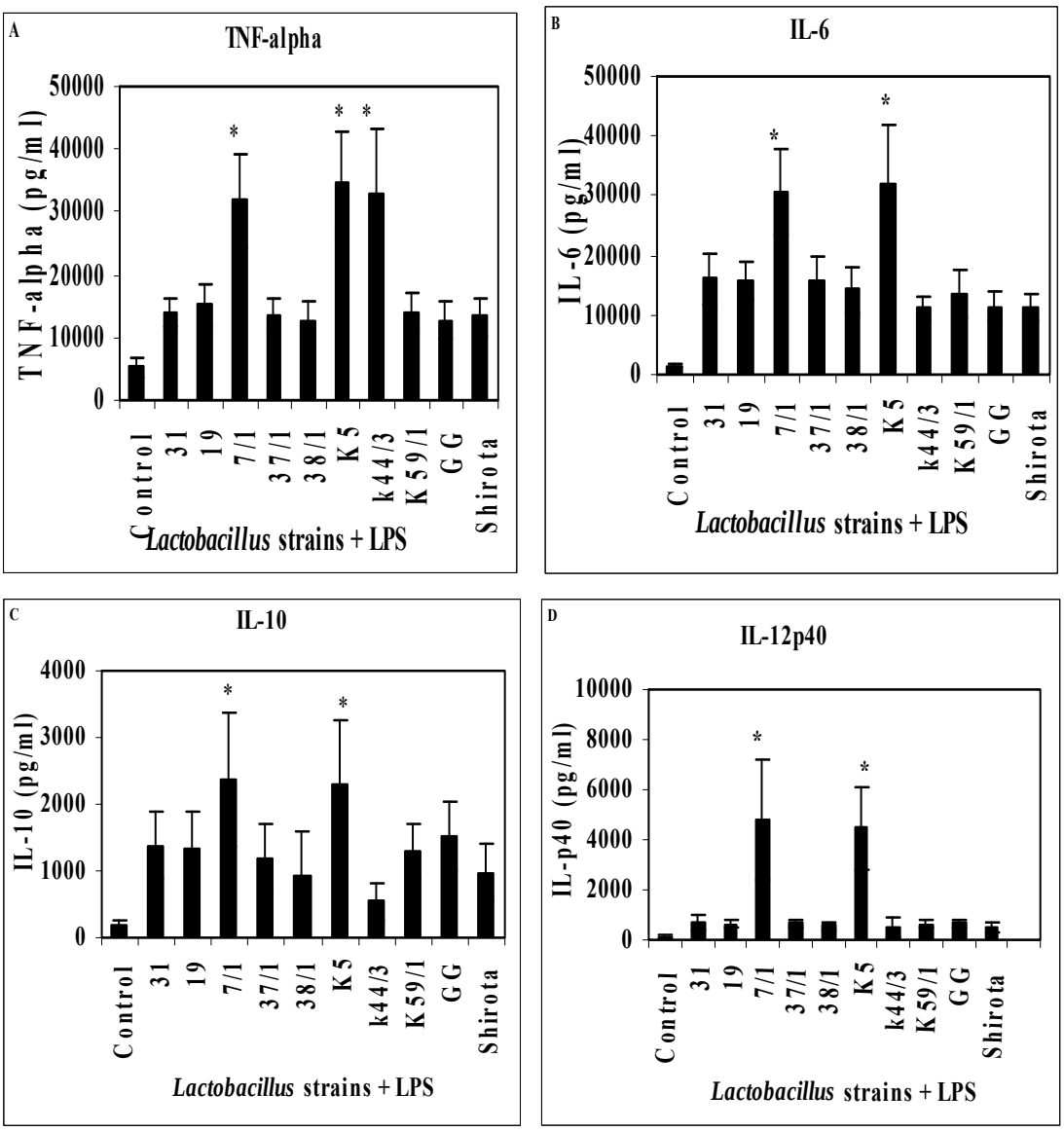

Figure 2. Cytokine production by murine macrophages J774.1cells (5x105 cell/well) after induction by LPS (100 ng/ml) and incubated with different heat-killed Lactobacillus strains (1x107 CFU/ml). Cytokines TNF-α (A), IL-6 (B), IL-10 (C) and IL-12p40 (D) were analyzed by ELISA kits.The results were expressed as average of three independent experiments and SD (error bar). Statistical significance of the differences in the level of cytokines secretion induced by Lactobacilli was determined by analysis of variance (ANOVA) using Friedman test (p< 0.00091.

DISCUSSION

Numerous investigations have been conducted on the effect of Lactobacilli on immune function as a criterion for selection of probiotics. However, interpretation of the resultant findings has in general; tended to be inclusive and even conflicting in the absence of clear mechanistics. Most studies have focused on the immunomodulatory effects of viable cells of lactic acid bacteria of their cell-wall extracts; however heat-killed Lactobacilli were used for the reason that using live cells might cause a bias in evaluating the immunomodulatory abilities of Lactobacilli. It has been known that not only live but also heat-killed LAB exhibit immunostimulatory activity [11-13]. Lyophilized live cells or even the nonlyophilized cells of each strain contain different populations of dead cells [14,15]. This population of dead cells could be even larger than of live cells, and this

situation could occur during the preparation procedures, depending on the viability of the strains [16]. This must affect the evaluation of the stimulatory ability when the dosage is controlled by the number of live cells. Therefore, dead cells were used to ensure that the total cells amount in all the strains was approximately the same; this would allow evaluation of their ability under the same condition. All heat-killed Lactobacillus strains were able to activate non stimulated or LPS-stimulated mouse macrophages to induce T cells toward Th1 immune responses in which two strains of Lactobacilli, Lactobacillus fermentum (7/1) and Lactobacillus fermentum (K5) produced the highest concentrations of TNF-α, IL-6, IL-10 and IL-12P40 compared to low levels induced by other strains. This capacity of Lactobacilli to variably induce cytokines indicates that species of Lactobacilli may differentially determine the immune responses. Macrophage recognition of and response to molecular structures on bacteria (pathogen-associated molecular patterns) occurs through a family of pattern recognition receptors designated Toll-like receptors (TLRs). Activation of these surface receptors by bacterial components are believed to be a key factor for recognition of the immune response and to mediate a link between the innate and the adaptive immune response [6].Differential activation of TLR by multiple pathogen-associated molecular patterns could induce different cytokine patterns. This fact might explain the observed differences in cytokine induction. TLRs have been found to be signal transducer for cell activation by peptidoglycan, lipoteichoic acid and bacterial lipoprotein in addition to LPS [17] However, as LPS also binds to TLR4 and CD14, these receptors constitute alternative activation pathways for LPS [18]. Other TLRs activated by the bacteria may be involved. Notably, no isolated bacterial component has been found entirely responsible for the potent induction of pro-inflammatory cytokines seen for whole bacteria, indicating that multiple receptors may be involved in mechanisms of stimulation [19]. Pro-inflammatory cytokines such as IL-12, TNF-α are among the first cytokines produced by phagocytic cells in response to encounters with pathogenic bacteria. Macrophage derived IL-12 stimulates INF-γ production in T cells and natural killer cells, which accelerates the development of native CD4+ T cells into Th1-type cells [20,12]. Therefore, IL-12 is a key immunoregulator favoring Th1-type responses. These results agree with those of Hessele and co-workers [22] who found that Gram-positive bacteria are, in general more potent IL-12 inducers than Gram-negative bacteria, while Christensen and co-workers [17] observed that Lactobacillus concentration giving rise to the highest level of IL-12 and TNF- α was much lower than the concentration inducing the highest level of anti-inflammatory cytokines such as IL-10.

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پوختە

کارتێکرنا ڕێکخستنا بەرهنگاربابەکتریا (Lactobacilli) لسەر شانێت (macrophages)

10 نێرێن جوری بەکتریا (Lactobacilli) هاتنە دەست نیشانکرن دناڤبەرا (110) تێرادا ئەوێن باشترین چالاکی دژی نەخوشیێن دەڤی نیشاندان.

ڤەکولین هاتە ئەنجامدان لسەر کارتیکرنا ڕێکخستنا بەرهنگاری ئەڤان تێرا لسەر شانێت (macrophages) ئەوێن نەهشیار یان هشیار کری بو کەرەستی (Lipopoly saccharide) .

ڤەکولینێ دیار کر کو جیاوازییێن بەرجاڤ ییّن هەین لسەر جورێ سایتوکاینێت ئەوێن هاتێنە ریشتین. هەمی تیرێن بەکتریایێ نیشاندا ڕێژا TNF-α:IL-10 ژ 24:1 بەلێ بتنێ تیرەکی چوری Lacto bacillus acidophilus (K44/13) بەرزترین ریژەدا ئەوژی110:1 وەئەڤە دیار بو کو ئەڤ تێرە بهێزترێن تێر بو یابەکتریا (Lactobacilli) بو رشاندنا سایتوکاینێیت هەودانێ .

الخلاصة

تاثير التنظيم المناعى بجراثيم (Lactobacilli) في الخلايا (macrophages) البلعمية الكبيرة نوع J 744.1

تم اختيار (10) عترة من بين (111) عترة من جراثيم(Lactobacilli) والتي اظهرت اعلى نشاط تضادي ضد ممرضات التجويف الفمي ودرست تأثيرها التنظيمي للمناعة في الخلايا البلعمية الكبيرة محفزة بمادة (Lipopoly saccharide) وغير المحفزة.

بينت الدراسة بوجود اختلافات معنوية في نمط المحركات الخلوية المفرزة بين العترات حيث اظهرت جميع العترات نسبة TNF-α:IL-10من1:24بينما

عترة واحدة (Lacto bacillus acidophilus K4413)اظهرت اقوى نسبة والتي كانت 1:110 وهذة تدل على ان هذة العترة هي الاقوى في تحفيز المحركات الخلوية ذات التأثير الالتهابي .

A NEW METHOD FOR TREATMENT OF DENTIN HYPERSENSITIVITY BY USING NANO FLUOR-HYDROXYAPATITE AND ND: YAG LASER: A SCANNING ELECTRON MICROSCOPIC STUDY

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Submitted 30 Jun 2012; accepted 3 Sep 2012

ABSTRACT

Objectives: The purpose of this study was to use a new method for treatment of dentin hypersensitivity and closure of dentinal tubules by combination of Nd:YAg laser and Nano Fluor-hydroxyapatite and compare the occluding effect of dentinal tubules of this type of treatment with other modalities like Cyanoacrylate and sodium fluoride by using scanning electron microscope.
Method: Sixty freshly extracted human premolar teeth had been collected randomly for this study. The coronal portion of each tooth was removed and the canals were instrumented and obturated with gutta percha. A 3 mm wide ring of root surface, 2 mm apical to the coronal rim of each specimen was cut by a rotary instrument attached to a special microlathe to expose underlining dentin. The teeth were divided randomly into six groups : Group 1: Ten teeth were treated with Nano Fluor – hydroxyapatite and Nd:YAG laser ,Group 2: Ten teeth were treated with Nd:YAG laser only, Group 3: Ten teeth were treated with Nano Fluor – hydroxyapatite only, Group 4: Ten teeth were treated with desensitizing cyanoacrylate resin bonding (Tetric N ceram), Group 5. Ten teeth were treated with 2% sodium fluoride, and Group 6: Ten teeth were not treated with any modality mentioned above (control group). Scanning electron micrograph with 5000X were taken to show the topography of each group after treatment.
Result and conclusion: The combination of Nano fluor hydroxyapatite and Nd:YAG laser is a promising treatment modality for dentin hypersensitivity and excellent method for closure of exposed dentinal tubules with significant difference when compared with other treatment modalities.

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Key words: Dentin Hypersensitivity ,Nano.Laser, Electeon Microscopy.

Dentin hypersensitivity is characterized by a sharp, localized and short pain in response to thermal, chemical, mechanical or osmotic stimuli, ceasing after their removal. The etiology of hypersensitivity is multifactorial, but the factors involved are not very clear [1] .
Several theories have been cited to explain the mechanism involved in dentinal hypersensitivity. The transducer theory, the modulation theory, the "gate" control and vibration theory, and the hydrodynamic theory have all been presented and discussed throughout the years. The latter, "hydrodynamic theory", developed in the 1960's and based upon two decades of research, is widely accepted as the cause of tooth sensitivity. Assumptions of the hydrodynamic theory conclude that when the fluids within the dentinal tubules are subjected to temperature changes or physical osmotic changes, the movement stimulates a nerve receptor sensitive to pressure, which leads to the transmission of the stimuli Based on the hydrodynamic theory, dentinal hypersensitivity is a transient tooth pain. The disease is characterized by a short, sharp pain arising from exposed dentin in response to a stimulus that cannot be ascribed to any other form of dental defect or pathology [2].
Therefore, in order to exhibit a response to the stimuli, the tubules would have to be open at the dentin surface as well as the pulpal surface of the tooth. The most

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important variable affecting the fluid flow in dentin is the radius of the tubuli. If the radius is reduced by one-half, the fluid flow within the tubuli falls to one-sixteenth of its original rate. Consequently, the creation of a smear layer or obliteration of the tubule can greatly increase the effectiveness of the treatment of this malady [3].

Various findings concerning the occluding effect of desensitizing agents on open dentinal tubules have been reported, but the problem of permeation through dentinal tubules has not been solved [4].

Sealing of dentinal tubules with resins and adhesives (cyanoacrylate) has been advocated for many years as a means of managing dentinal hypersensitivity. In general, results have been good but problems arise when the adhesive breaks away resulting in exposure of the tubules. This technique is generally reserved for cases of specific and localized dentinal hypersensitivity rather than generalized dentinal pain [3].

Many clinical studies have shown that treatment of exposed root surfaces with sodium fluoride toothpaste and concentrated fluoride solutions is very efficient in managing dentinal hypersensitivity [5].

The improvement appears to be due to an increase in the resistance of dentine to acid decalcification as well as to precipitations in the exposed dentinal tubules. Tal et al., suggested that the probable desensitizing effects of fluoride are related to precipitated fluoride compounds mechanically blocking exposed dentinal tubules or fluoride within the tubules blocking transmission of stimuli [6].

HydroxyapatiteHA (Ca10(PO4)6(OH)2) has attracted much interest as an implant material for teeth and bones due to the similarity of its crystallography and chemical composition to that of human hard tissues . In most cases, the controlled solubility of HA is an important factor, since it induces bioactivity, osteoconductivity, and therapeutic effects. On the other hand, pure fluorapatite (FA) with a chemical formula, Ca10(PO4)6(F)2 is thought to have a much lower solubility than HA due to greater chemical and structural stability [7].

Moreover, the FHA (Ca10(PO4)6(OH,F)2) forms solid solutions with HA by the replacement of OH− by F−. Hence, modulation of the extent of fluoride substitution provides an effective way of controlling the solubility of the apatite. In addition, F− enhances the mineralization and crystallization of calcium phosphate compounds during the bone regeneration [8].

Laser technology has been used in many dental applications. When laser is used at a level of 150—190 millijoules (mJ), the dentine is not only vaporized, but it also melts and closes off partially the dentinal tubules. Chiang et al., demonstrated that the use of mesoporous silica biomaterial, which contain Nano-sized calcium oxide particles(40 nm), can penetrate through dentinal tubules with a depth of 100 μm and significantly reduce dentin permeability. Thus, using Nano sized particles has great potential applications for the clinical treatment of dentin hypersensitivity [9].

To the Researchers knowledge, there are no published papers available concerning the use of Nano Fluor –hydroxyapatite with laser as a treatment modality for dentin hypersensitivity. Accordingly, this study design will be show the effect of using the combination of Nd:YAg laser and Nano Fluor-hydroxyapatite in closing the dentinal tubules and treating dentin hypersensitivity and compare this type of treatment with other modalities by using a scanning electron microscope.

METHODS

Sample selection

Sixty freshly extracted human premolar teeth had been collected randomly for this study. The age range was

between 18 – 30 years. Sex, pulpal status or reasons for extraction are not recorded.

Sample preparation

After extraction, all teeth were stored in normal saline solution at room temperature. External soft tissue and debris were removed using an ultrasonic scaler. The coronal portion of each tooth was removed to the level of the cervical line using a diamond disc with a straight handpiece.

The patency of each canal was established by passing a no. 10 k-type file through the apical foramen, the pulpal tissue was removed by using barbed broaches, and the working length was determined by subtracting 1 mm from the length at which the tip of the no.10 file just appeared at the apical foramen.

The canals were instrumented using a conventional hand instrumentation technique with circumferential filing action to master apical file no. 60 k-type files. Each canal was irrigated after each instrument size with 2.5% sodium hypochlorite solution throughout the canal preparations, and the canals were dried with paper points. Master gutta percha cones were fitted to within 1 mm of the working length. The canals were obturated completely using the lateral condensation technique (using zinc oxide type sealer).

Excess gutta percha was removed with a heated instrument to a level 2 mm apical to the orifices of the canals, and then the coronal access preparations were closed with zinc phosphate cement. All teeth were stored in 100% humidity at 37℃ for 48 h to allow time for the sealer to set. A 3 mm wide ring of root surface, 2 mm apical to the coronal rim of each specimen was cut by a rotary instrument attached to a special microlathe to expose underlining dentin [10].

Sample grouping

The teeth were divided randomly into six groups:

Group 1. Ten teeth were treated with Nano Fluor – hydroxyapatite and Nd:YAG laser (focus mode at a wavelength of 1064 nm, 100 mJ, 100 Hz repetition rate for 2 second) .

Group 2. Ten teeth were treated with Nd:YAG laser only (focus mode at a wavelength of 1064 nm, 100 mJ, 100 Hz repetition rate for 2 second) .

Group 3. Ten teeth were treated with Nano Fluor – hydroxyapatite only.

Group 4. Ten teeth were treated with desensitizing cyanoacrylate resin bonding (Tetric N ceram).

Group 5. Ten teeth were treated with 2% sodium fluoride.

Group 6. Ten teeth were not treated with any modality mentioned above (control group).

The teeth from Group one, the exposed dentinal tubules were swiped with Nano Fluor –hydroxyapatite and subjected to the Nd:YAG laser (focus mode at a wavelength of 1064 nm, 100 mJ, 100 Hz repetition rate for 2 seconds) . The teeth from the second testing group were treated with Nd:YAG laser only(focus mode at a wavelength of 1064 nm, 100 mJ, 100 Hz repetition rate for 2 seconds) . The teeth from the third testing group were treated with Fluor -hydroxyapatite by applying a single layer on the surface of exposed dentinal tubules. The teeth from the fourth group were treated with a cyanoacrylate adhesive system (Tetric N ceram) by applying a single layer on the surface of exposed dentinal tubules. While the teeth from the fifth group will be treated with a single application of 2% sodium fluoride. The sixth group will be left without treatment as control group.

Scanning electron microscope examination

The teeth were air-dried and mounted on aluminum stubs. After sputtering with a 40 nm layer of gold by using Balzers SCD 050 apparatus, wall surfaces of the treated samples were examined using a scanning electron microscope (5000X) operating at 10—15 kV. This showed the surface topography of the dentine substrate after

treating with different modalities of dentin hypersensitivity [11].

Photomicrographs were taken from each dentinal surface examined, approximately 100 µm above the gingival margin, at X5000 magnification.

Percentage of occluded tubules was obtained by dividing the total number of occluded tubules by the total number of tubules in each photomicrography. This result was then multiplied by 100, to obtain the percentage of occluded tubules for each photography. Results were statistically analyzed by using Tukey test ($p < 0.05$) .

RESULTS

The data from (Table 1) and (Figure 1) indicated that the highest occluding effect for dentinal tubules was for Nano fluor hydroxyapatite and Nd:YAG laser (99.8%). While other treatment modalities showed similar occluding effect on dentinal tubules. The Tukey test explained significant differences in occluding effect between (Nano fluor hydroxylapatite / Nd:YAG laser) and other treatment modalities. While there were no significant differences among group 2 , 3, 4 and 5. There was highly significant differences between control group and other five groups. (Table 2)

Scanning electron micrograph for specimens of Group 1 (Nano fluor hydroxyapatite and Nd:YAG laser) showed many deposits on the dentinal surface
in and around the orifices of the dentinal tubules and recrystallization of dentin substrate with Nano fluor hydroxyapatite (Figure 2).

Group 2 (Nd:YAG laser)) showed partial deposits on the dentinal surface and some orifices of dentinal tubules that remained patent(Figure 3) The scanning electron micrograph for specimens of Group 3 showed deposition of Nano fluor hydroxyapatite on the dentin surface but the dentinal tubules not completely closed (Figure 4). Group 4 showed the scanning electron micrograph of dentin after treatment with dentin adhesive system (Tetric N ceram). There was a partially closure of some dentinal tubules but the other still patent (Figure 5).

Group 5 showed the scanning electron micrograph of dentin after treatment with 2% sodium fluoride. The dentinal tubules are not completely closed (Figure 6). In the group 6 (control group) the dentinal tubules looked open with some deposit of smear layer around the orifices of the tubules (Figure 7).

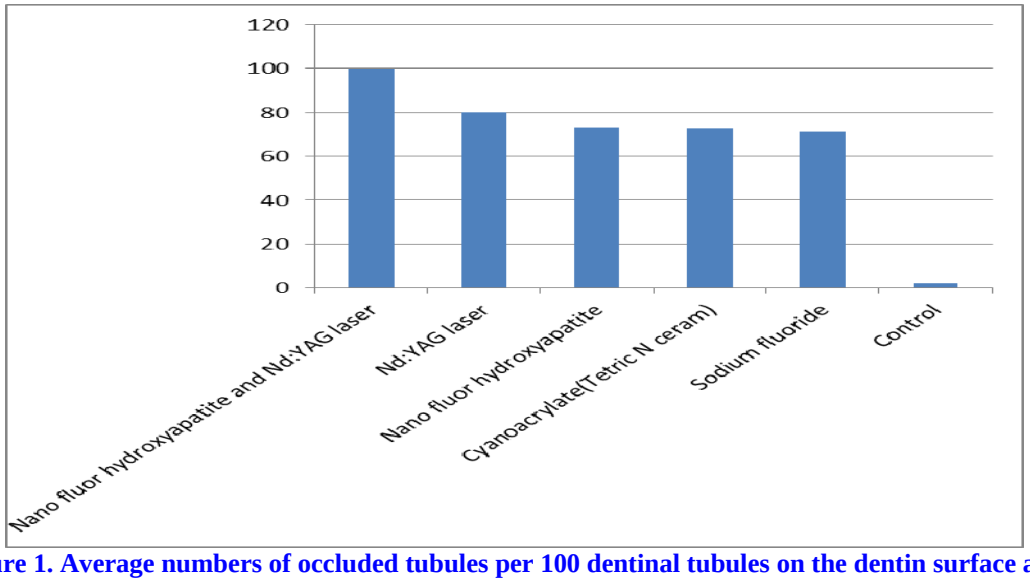

Figure 1. Average numbers of occluded tubules per 100 dentinal tubules on the dentin surface after different treatments.

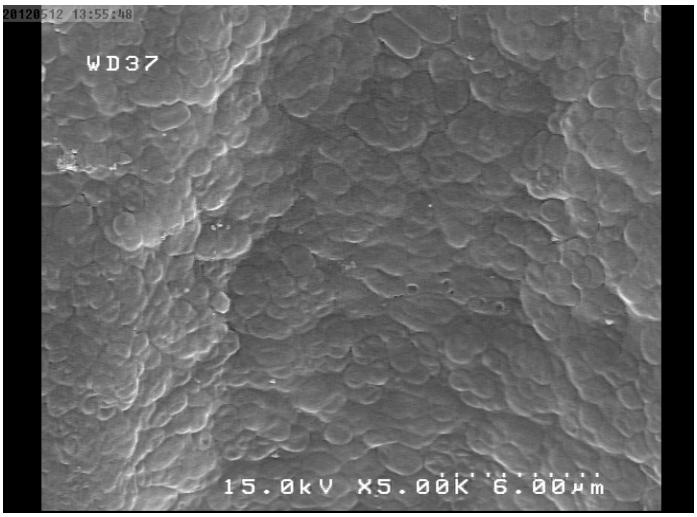

Figure 2.Scanning electron micrograph of dentin after treatment with Nano fluor hydroxyapatite and Nd:YAG laser (X5000).

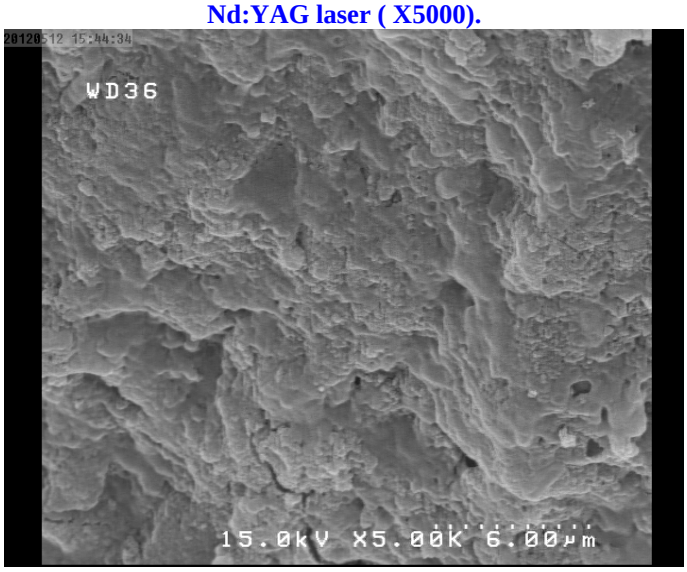

Figure 3. Scanning electron micrograph of dentin surface treated with Nd:YAG laser (X5000).

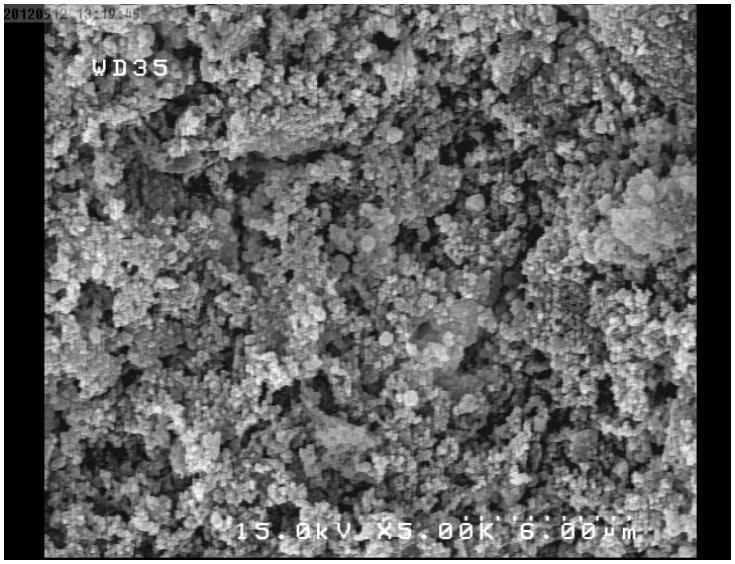

Figure 4.Scanning electron micrograph of dentin surface treated with Nano fluor hydroxyapatite (X5000).

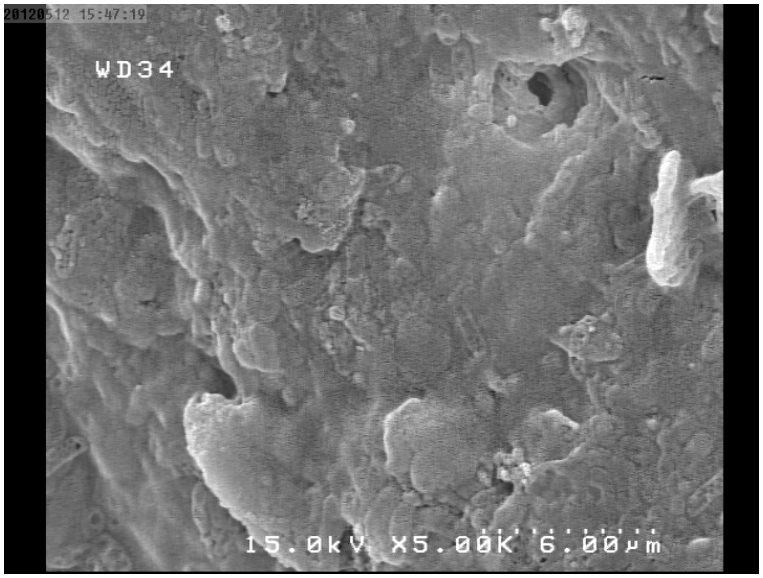

Figure 5. Scanning electron micrograph of dentin surface treated with dentin adhesive system (Tetric N ceram) (X5000).

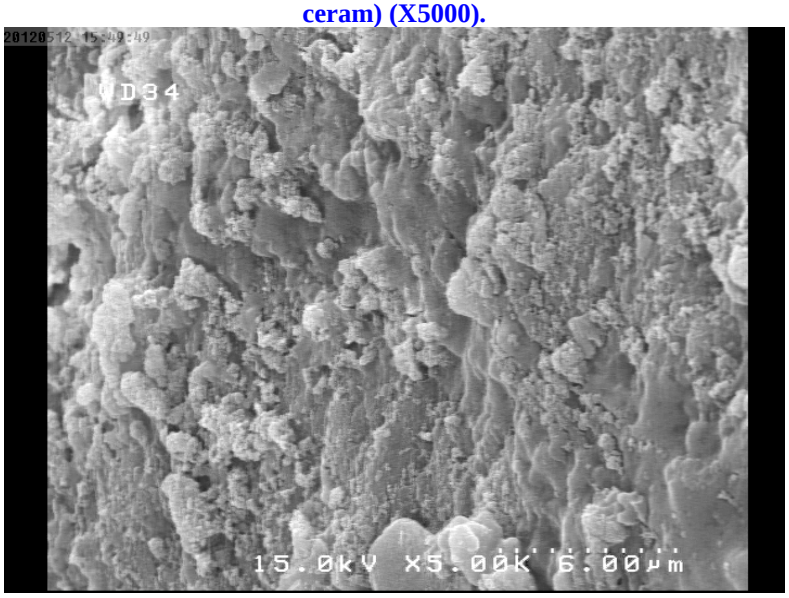

Figure 6. Scanning electron micrograph of dentin surface treated with sodium fluoride (X5000).

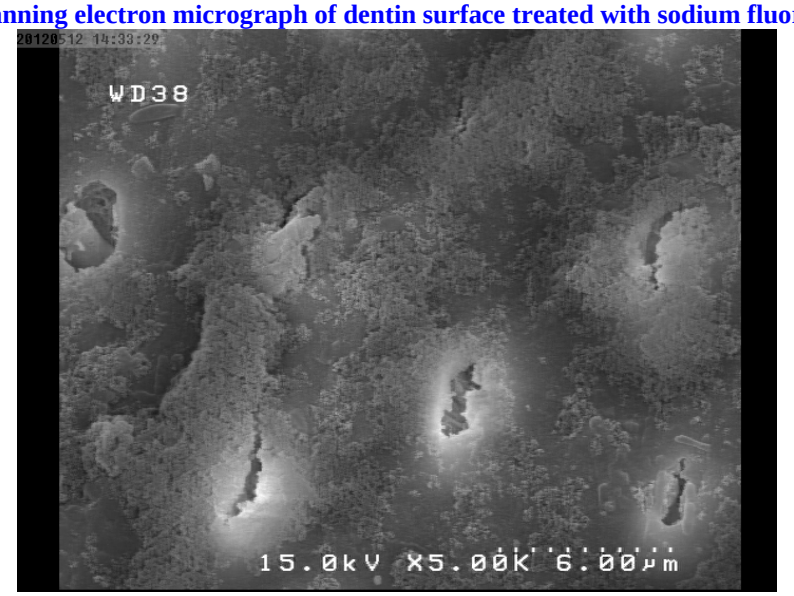

Figure 7. Scanning electron micrograph of dentin surface without any treatment (X5000).

Table 1. Average numbers of occluding tubules per 100 dentinal tubules on the dentin surface after different treatments.

Treatment	Mean %	Standard deviation ±
Nano fluor hydroxyapatite and Nd:YAG laser	99.8	±3.3
Nd:YAG laser	83.1	±5.2
Nano fluor hydroxyapatite	82.3	±4.4
Cyanoacrylate(Tetric N ceram)	82.1	±7.2
2% Sodium fluoride	81.4	±3.5
Control	2.1	±0.7

Table 2. Tukey test to show the significance differences among groups.

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Group 1		Sig.	Sig.	Sig.	Sig.	H. Sig.
Group 2	Sig.		N. Sig.	N. Sig.	N. Sig.	H. Sig.
Group 3	Sig.	N. Sig.		N. Sig.	N. Sig.	H. Sig.
Group 4	Sig.	N. Sig.	N. Sig.		N. Sig.	H. Sig.
Group 5	Sig.	N. Sig.	N. Sig.	N. Sig.		H. Sig.
Group 6	H. Sig.	H. Sig.	H. Sig.	H. Sig.	H. Sig.	

N. Sig. (non-significant P≥0.05), Sig. (significant P<0.05), H. Sig. (highly significant P<0.01)

DISCUSSION

Dentinal tubule occlusion can contribute to the reduction of hypersensitivity. This study sought to evaluate in vitro agents that are utilized in treating dentin hypersensitivity, as they can affect dentin permeability and dentinal tubule occlusion. The desensitizing agents which have been used in this study are cyanoacrylate (Tetric N ceram) and 2% sodium fluoride; these products were chosen based on their popularity and their relative effectiveness. While Nd:YAG laser was selected for this study due to its availability and affectivity. Nano Fluor – Hydroxyapatite are recently prepared for treatment of dentin hypersensitivity. Some researchers used a combination of different desensitizing agents to treat dentin hypersensitivity but they didn't use the combination of Nd:YAG laser and Nano Fluor hydroxyapatite for this purpose.

In the present study, 2% sodium fluoride and cyanoacrylate (Tetric N ceram) were unable to reduce dentin permeability or obliterate dentinal tubules. Fluorine and cyanoacrylate have high solubility in water and thus could have been removed during sample washing. A number of studies have shown that fluoride does not affect dentinal tubules. Conversely, two studies published years ago, demonstrated that sodium fluoride was able to reduce dentin permeability. Fluoride dentifrice promotes dentinal tubule occlusion; however, Its concentration might have been the primary cause [12, 13].

The result of present study comes in disagreement with Maria et al who indicated that using 2% sodium fluoride is

effective method for inducing dentinal tubule occlusion completely [14].

Nd:YAG laser seemed to produce occlusion for some of dentinal tubules due to the recrystallization of dentin and bending of the inner walls of the orifices of these tubules to the inside direction and this came in agreement with Al Azawi and Dayem [15].

Although the occlusion of dentinal tubules by Nd:YAG laser gave good result but it was still not enough to obliterate all of the dentinal tubules. Addition of Nano fluor hydroxyapatite might solve this drawback. The Nano fluor hydroxyapatite particles deposited inside dentinal tubules and around them, and by using laser, these deposits would melt and fuse with the inner surfaces of dentinal tubules and form excellent plug. Therefore, the result of the first group indicated almost complete closure and obliteration of dentinal tubules. This might lead to overcome dentin hypersensitivity problem. The first group showed a significant difference in the occluding of dentinal tubules in comparison with other groups. As a conclusion for the present study, the combination effect of Nano fluor hydroxyapatite and Nd:YAG laser was a promising treatment modality for dentin hypersensitivity and excellent method for closure of exposed dentinal tubules.

CONCLUSION

The combination of Nano fluor hydroxyapatite and Nd: YAG laser is a promising treatment modality for dentin hypersensitivity and excellent method for closure of exposed dentinal tubules with significant difference when compared with other treatment modalities.

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پوختە

ڕێکا جارەسەرکرنا ناسکیا دژوار یا دنتینا ددانا بڕێکا کارئینانا نانوفلور– هیدروکسی أباتیت وYAG :Nd لیزر : تۆێژینەوەکا ڤەمالینا مایکروسکوبێ ئەلیکترونیك

ئارمانج: ئارمانج ژ ڤێ تۆێژینەوێ بکار ئینانا ڕێکەکا نی بوو ژبو جارەسەرکرنا ناسکیا دژوار یا دنتینا ددانا و گرتنا بوریێت دنتینێ ژلایێ گروپەکێ YAG :Nd لیزر ونانوفلور– هیدروکسی أباتیت و وبەراوردکردنا وێ وهەلسەنگاندن لگەل گرتنا بوریێت دنتینێ ێت ڤی جورێ جارەسەرکرنێ لگەل ڕێکێت دی ێت جارەسەریێ وەك cyanoacrylates وفلوراید صودیوم بڕێکا کارئینانا ڤەمالینا مایکروسکوبێ ئەلیکترونیك .

ڕێکا تۆێژینەوێ: شێست ژ ددانێت مروڤا ێتب بھ ری جیتنێ ھاتنە کێشان لدەمەکێ نیزیك و ھاتنە کوم ڤەکرن بڕێکەکا جاڤ گرتی ڤە بو ڤێ تۆێژینەوێ سەرێ ھەر ددانەکی ھاتە برین و پاش رھێت ددانا ھاتنە پاقژکرن و ھاتنە گرتن ب گەتا پێکا.

3 mm فرەھی ژ لایێ رھا ددانی و 2mmژ لایێ سەرێ ددانی یا ھەر ددانەکی ھاتنە برین بڕێکا ئامیرەکێ زفروك کو گرێدای ب مایکرولیس ڤە دا کو دنتینا د بنیدا بدەرکەڤیت .ددان ب جاڤ گرتیڤە ھاتنە دابەش کرن لسەر شەش گروبا:

گروب 1 : 10 ددان ھاتنە جارەسەرکرن ب نانوفلور– ھیدروکسی أباتیت وYAG :Nd لیزر ,گروب 2: 10 ددانا ھاتنە جارەسەرکرن ب YAG :Nd لیزر ب تنێ ,گروب 3 : 10 ددان ھاتنە جارەسەرکرن ب نانوفلور– ھیدروکسی أباتیتب تنێ ,گروب 4: 10 ددان ھاتنە جارەسەرکرن ب دەرمانێ دژی ناسکیێ (Tetric N ceram) cyanoacrylate resin bonding,گروب 5: 10 ددان ھاتنە جارەسەرکرن ب 2٪ سودیوم فلوراید ,گروب6: 10 ددان نە ھاتنە جارەسەر کرن ب ج ژ ئەڤێت سەری ھاتینە گوتن وەك گروپێ کنترولێ مان.

دەر ئەنجامێت کوتایی و پوختە: تێکەلکرنا ھەردوو نانوفلور– ھیدروکسی أباتیت وYAG :Nd لیزر دەرکەت کو ھاندەرە وپاشە روژەکا باش ھەیە ژ بو جارەسەرکرنا ناسکیا دنینێ و ژ بو گرتنا بوریێت دنتینێ تشتەکێ ھەرە باشە لگەل جوداھێت گەل دیار و مەزن دەمی براورد دکەین لگەل جوریێت دی ێت جارەسەرکرنێ.

الخلاصة

طريقة جديدة لعلاج فرط الحساسية العاج باستخدام النانوفلور– هيدروكسي أباتيت وYAG :Nd ليزر: دراسة مجهرية الإلكتروني الماسح

خلفية وأهداف البحث: كتابة الوصفة هي علم وفن في آن واحد حيث تعكس رسالة الواصف (الطبيب) للمريض. كتابة الوصفة هي من اهم المبادئ الاساسية التي يحتاجها الطبيب. ان هدف الدراسة هو لاجراء مسح للوصفات الطبية (التي كتبت من قبل الاطباء) للعناصر الاساسية للوصفة.

الأهداف: وكان الغرض من هذه الدراسة هو استخدام طريقة جديدة لعلاج فرط الحساسية العاج وإغلاق الأنابيب العاجية من قبل مجموعة من YAG :Nd ليزر والنانوفلور– هيدروكسي أباتيت ومقارنة تأثير انسداد الأنابيب العاجية من هذا النوع من العلاج مع غيرها من الطرق مثل cyanoacrylates وفلورايد الصوديوم باستخدام المجهر الإلكتروني الماسح.

المنهجية: تم استخراج ستين سناً من أسنان قبل الطواحن البشري حديثا والتي جمعت عشوائيا لهذه الدراسة. وتمت إزالة جزء من الإكليلي لكل سن وكانت القنوات المجهزة وobturated مع gutta percha. وتمت عمل حلقة 3 مم واسعة من سطح الجذر، وقطع القمي 2 مم إلى حافة الاكليلية من كل عينة من أداة دوارة مثبتة في microlathe الخاصة مؤكدا لتعرض العاج. وتم تقسيم عشوائي للأسنان إلى ست مجموعات: وكل مجموعة تتألف من عشرة اسنان المجموعة 1: تم علاج الأسنان العشرة مع النانوفلور– هيدروكسي أباتيت وYAG :Nd ليزر ، المجموعة 2: تم علاج الأسنان العشرة مع YAG :Nd ليزر فقط، المجموعة 3: تم علاج الأسنان العشرة مع النانوفلور– هيدروكسي أباتيت فقط، المجموعة 4: تم علاج الأسنان العشرة مع مزيل للتحسس CYANOACRYLATE الترابط الراتنج (N Tetric CERAM)، المجموعة 5 :وعولج عشرة أسنان بفلورايد الصوديوم 2٪، ومجموعة 6: لم يعاملوا عشرة أسنان مع أي طريقة المذكورة أعلاه (المجموعة الضابطة).

نتيجة وخاتمة: مزيج من النانوفلور– هيدروكسي أباتيت وYAG :Nd ليزر هو طريقة واعدة لعلاج فرط الحساسية العاج و وسيلة ممتازة لإغلاق الأنابيب العاجية مع اختلاف كبير بالمقارنة مع طرق العلاج الأخرى.

GENETIC POLYMORPHISMS OF P21 AND RISK OF PROSTATE CANCER

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ABSTRACT

P21 is a cyclin-dependent kinase inhibitor that is pivotal in arresting cellular growth, in terminal differentiation and in apoptosis. Two polymorphisms in the p21Waf1/Cip1gene, i.e., codon 31 in the coding region and 3'UTR, have been identified and appeared to influence the expression of p21Waf1/Cip1. Several epidemiologic studies have examined the effect of these polymorphisms on cancer risk, which has motivated us to study the relationship between p21 codon 31 and 3'UTR polymorphism and the risk of prostate cancer among a sample population of Kurdistan province of IRAN. Genotyping was done by PCR-RFLP using DNA from (i) 45 prostate cancer paraffin embedded tissues, and (ii) a total of 45 normal blood samples. The PCR-RFLP products were 169bp for C allele, 115bp and 54bp for A allele in codone 31 and were 191 bp for C allele, 76bp and 115bp for T allele in 3'UTR. Frequencies of C/C and C/A plus A/A genotypes in 31 codone, were 31 (68.8%), 14 (31.2%) in carcinoma cases and 18 (60.0%), 12 (40.0%) in control cases, respectively. Allelic frequencies for controls were 0.866 for C and 0.124 for A and for patients were 0.84 and 0.16 for C and A respectively. For 3'UTR the only observed genotype were C/C for both control and cases. Although the sample size is relatively small, these findings suggest that a codon 31 polymorphism in p21 may be associated with the development of prostate cancer.

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Key words: P21 codon 31; Prostate cancer; Genetic polymorphism; PCR-RFLP

The p21 (also known as Waf1/Cip1/CDKN1A) protein is a CDK inhibitor that is essential for cellular growth, differentiation and apoptosis [1]. p21, a putative tumor suppressor gene, is located on chromosome 6p21.2 and encodes a 21-kDa protein [2] that belongs to the CIP/KIP family, which includes p27 (1) and p57 [3,4]. The CIP/KIP proteins share some common sequence motifs that mediate interaction between CDK inhibitors and cyclin-CDK complexes [5,6]. The expression of p21 itself is up-regulated by p53 in response to DNA damage, leading to either cell-cycle arrest at the G1 checkpoint or apoptosis [1] p21 expression can suppress tumor growth by inhibiting PCNA-dependent DNA replication and mismatch repair in vitro [7,8], and increased expression of p21 and the accompanying reduction in overall CDK activity are associated with cell differentiation [9]. Somatic mutations in the p21 gene are rare in human malignancies [10], but reduced p21 expression in tumors has been associated with poor prognosis in humans [11-13], including patients with laryngeal SCC [13]. It is, therefore, likely that genetic variants in p21 may modulate its expression and thereby affect carcinogenesis. A total of 40 polymorphisms of p21 have been identified (available at http://egp.gs.washington.edu) [(14)], of which 35 are intronic. Sun and his colleagues [(15)] reported a polymorphism in the p21 codon 31 that produces a C to A transversion and causes a substitution from serine (ser) to arginine (arg). Subsequently, a series of epidemiologic studies have examined the effect of this polymorphism on lung cancer, cervical cancer, breast cancer and nasopharyngeal cancer with conflicting results [16-22]. The ser allele has been suggested to be a risk factor for cancers in breast and cervix [17, 18], whereas the arg allele has been associated with the

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development of lung cancer [16]. Since no one to our knowledge has investigated the polymorphic effect of the p21 codon 31 on prostate cancer risk, we decided to pursue such an inquiry.

METHODS

Patient Population/Tissue Samples: A total of 45 patients with prostate cancer were evaluated. Medical records of the patients were reviewed to obtain age at the time of surgery, surgical methods used to obtain the sample (s), and pathological features of the tumor. Archival formalin-fixed, paraffin-embedded tissue was obtained from the pathology files of the Tohid Hospital- Sanandaj. Tumors were histologically verified as prostate cancer. Serial 20-µm-thick sections were cut from each tissue block, and were used for micro dissection to isolate DNA for PCR. Blood samples from 45 cancer free controls were randomly selected from a pool of healthy volunteers who visited the clinical laboratory of Dr. Verdi- Sanandaj, and used for DNA extraction.

DNA extraction: Areas of prostatic carcinoma in either primary or metastatic tissue, identified histologically on an adjacent hematoxylin and eosin-stained tissue section, were microdissected with sterile scalpel blades into 1.5-ml plastic Eppendorf tubes from one to two 20-µm-thick unstained sections. A fresh scalpel blade was used for each sample. Paraffin was removed by two extractions with xylene followed by two extractions with cold 100% ethanol. Specimens were resuspended in 500 µl lysis buffer containing proteinase K and incubated over night at 55˚C. Extracted DNA qualified by agarose gel electrophoresis. Specimens were centrifuged at 13,000 X g for 15min and stored at -20˚C. Approximately 3 µl of the supernatant were used directly in the initial PCR reactions. Genomic DNA was extracted from whole blood samples of controls, using salting out DNA extraction method.

PCR-RFLP: The status of the p21 polymorphism in codon 31 and 3'UTR was determined by PCR-RFLP. The amplification primers for the 169 bp region in exon 2 of the P21 gene were: 5'- CCTTCCTTGTATCTCTGCTG - 3' (forward) and 5'- CTCACGGGCCTCCTGGAT -3' (reverse). The amplification conditions for codon 31 were: initial denaturation at 94°C for 4 minutes, followed by 35 cycles at 95°C for 30 seconds, 58°C for 45 seconds, 72°C for 45 seconds, and final 7 minute extension step at 72°C. The 169 bp PCR amplified fragment of p21 exon 2 was subsequently digested with the Bpu1102I restriction enzyme. Digestion of the wild-type allele C created DNA fragments of 54 and 115 bp, whereas the A allele, which lacks a Bpu1102I site, yielded the original 169 bp fragment. The amplification primers for the 3'UTR region of the P21 gene were: 5'- CTCA GATTTCTACCACTCCA - 3' (forward) and 5'- ACACCTCCTCATACATACC-3' (reverse). The amplification conditions was as above with exception in annealing step which was 59°C, instead of 58°C for 30 seconds. The 191 bp PCR amplified fragment of p21 3'UTR was subsequently digested with the pstI restriction enzyme. Digestion of the wild-type allele C created DNA fragments of 76 and 115 bp whereas the T allele, which lacks a pstI site, yielded the original 191 bp fragment. The PCR product was subjected to electrophoresis on a 2% agarose gel and stained with ethidium bromide. The restriction digestion product was subjected to 8% PAGE.

Statistical analysis: Hardy–Weinberg equilibrium was tested by comparing expected and observed genotype frequencies by $\chi 2$ test. Using multiple logistic regression models, we assessed the association of p21 codon 31 and 3'UTR polymorphism with prostate cancer risk overall. Data were analyzed using the Popgene32 software.

RESULTS AND DISCUSSION

Whole-mount specimens of the prostate gland containing multiple foci of cancer (an average of three per gland) were available from 45 patients with metastatic disease who had undergone a radical prostatectomy. The age of patients ranged from 22 to 90 years (Figure 1). To detect mutations within the DNA sequence of the p21 gene, serial 20-µm-thick sections were cut from each tissue block, were used to isolate DNA for PCR, and amplified codon 31 region exon 2 and 3' UTR with specific primers (Figure 2). The status of the p21 C98A and C70T polymorphisms were determined by PCR-RFLP (Figure 3).

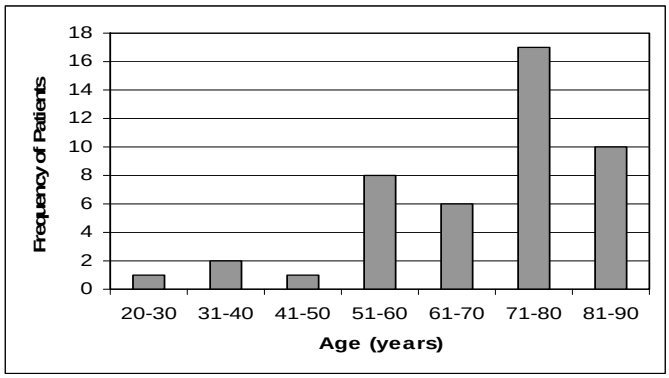

Figure 1 . Age distribution of Prostate Cancer Patients, which have been selected for this study.

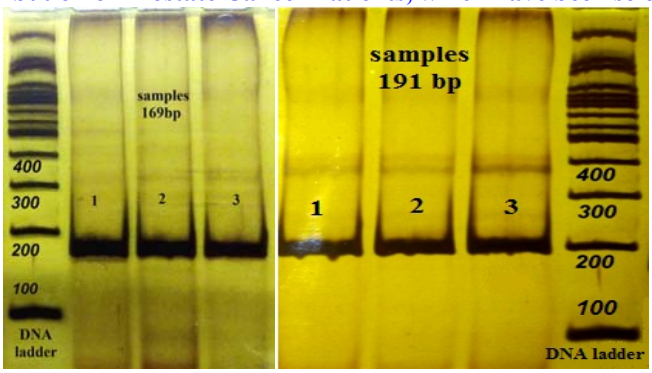

Figure 2. Polyacrylamide gel electrophoresis (8%) analysis and qualification of the specimens at DNA extraction stage. (Left) Exon 2 codon 31 (Right) 3' UTR.

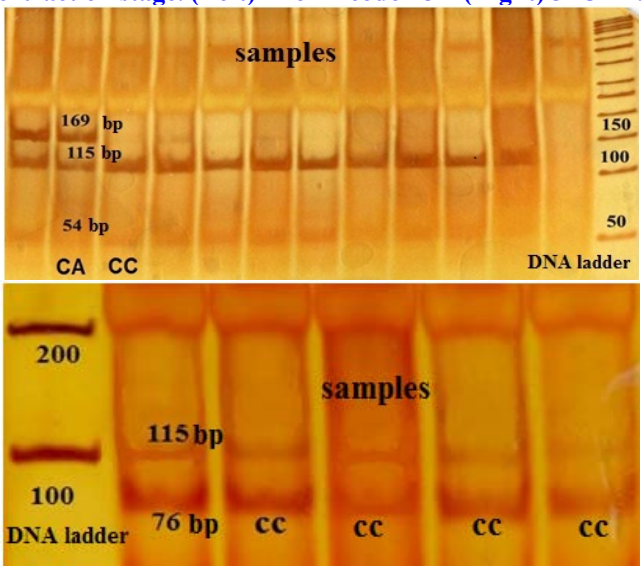

Figure 3 .(Above) PCR-RFLP analysis of the p21 C98A polymorphism. From left Lanes 1and 2: heterozygote (C/A genotype); lanes 3-11: homozygote C/C genotype; lane 13: Molecular weight marker. (Below) PCR-RFLP analysis of the p21 C70T polymorphism. From left Lanes 1: Molecular weight marker; other lanes : homozygote C/C genotype.

The PCR-RFLP products were 169bp for A allele, 115bp and 54bp for C allele. Frequencies of C/C and C/A plus A/A genotypes were 31 (68.8%), 14 (31.2%) in carcinoma cases and 18 (60.0%), 12 (40.0%) in control cases, respectively (Table I).

Table 1. Comparison of genotypes (codon 31) between prostate cancer patients and controls.

Genotype Sample	C/C	C/A & A/A
Control	73.4%	26.4%
Patients	68.8%	31.2%

Allelic frequencies for controls were 0.866 for C and 0.124 for A, and for patients were 0.84 and 0.16 for C and A respectively (Table 2).

Table 2. Allelic frequency in codon 31 for control and prostate cancer specimens.

Allele Sample	C	A
Control	0.866	0.266
Patients	0.84	0.16

A similar study on Taiwanies population showed different results [23] which ethnic differences and size of the sample population may be the main factor for that differences. In another study on the esophageal squamous cell carcinoma didn't show any correlation between p21 polymorphism and that cancer [24]. Frequencies of C/C and C/T plus T/T genotypes were 45 (100%) and 0 (0.00%) in carcinoma cases, versus 30 (100.0%) and 0 (0.00%) in control cases, respectively (Table 3).

Table 3. Comparison of genotypes (3' UTR) between prostate cancer patients and controls.

Genotype Sample	C/C	C/T & T/T
Control	100%	0%
Patients	100%	0%

Allelic frequencies for controls were 1.00 for C and 0.00 for A, and for patients were 1.00 and 0.00 for C and A respectively (Table 4).

Table 4. Allelic frequency in 3' UTR for control and prostate cancer specimens.

Allele Sample	C	T
Control	1.00	0.00
Patients	1.00	0.00

Li et al in 2005 [25] also evaluated together by the number of risk alleles, there was a significant increase in SCCHN risk that was dependent on the number of risk alleles, and suggested that the presence of these two polymorphisms may be a marker of genetic susceptibility to SCCHN. From the other hand Taghavi et al study on the relationship between these two polymorphism and ESCC, suggested that these two p21 polymorphisms, both alone and in combination, are not genetic susceptibility biomarkers for ESCC. However, their interaction with cigarette smoking may influence the susceptibility to ESCC development in northeastern Iran (24). We should note that the codon 31 of p21 gene is located in a zinc finger motif of the gene product [26], protein p21 ,and the above mentioned polymorphism replaces ser for arg in p21 protein, which may be deeply alter the structure as well as the function of the p21 protein. The $\chi 2$ test used to evaluate any association between p21 polymorphisms and prostate cancer (Table 5).

Table 5. Results of $\chi 2$ test and contingency for patients and control specimens.

Allele Sample	Frequency	χ^2	Contingency ($p<0.05$)
Control	45	0.37	0.54
Patients	45	1.4	0.23

$\chi 2$ for both cancerous and control populations were bigger than 0.05, so both of those populations have hardi-weinberg equilibrium. Results of heterozygosity analysis for exon 2 (Table 6) shows that difference between expected and observed

heterozygosity for both test and control populations are less than 0.1, also the difference of expected and observed heterozygosity between test ant control populations is negligible so this results confirm the exon 2, as a polymorphic site. Although the sample size is relatively small, these findings suggest that a codon 31 polymorphism in p21 may be associated with the development of prostate cancer ($p<0.05$). However the results for 3'UTR region did not indicate any association between its polymorphism and prostate cancer in that population.

Table 6. Results for expected and observed heterozygosity in patient and control populations.

Population	Observed heterozygosity	Expected heterozygosity
Control	**0.177**	**0.163**
Patients	**0.311**	**0.262**

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پوخته

پیوه ندی نیوان چه ند شیوەیی جینیp21 و ئه گەری توش بوونی شیرپەنجەی پروستات له نیو بەشیك له دانیشتووانی پارێزگەی کوردستانی ئێران

پروتئینی p21 وەك لەغاویکە بو کاینازی به ستراوە به سایکلین کە وا، لە راوەستاندنی گەشەکردنی خانەکان، جیاکاری دووایی خانەکان، و مەرگی دیاریکراوی ئەوان دا دەوریکی زور گرینگی هەیە دوو جور له چەند شیوەیی بو جینیp21 ناسراون کەوا کاریگەریان هەیە لەسەر خودەرخستنی جینیp21. یەکەمیان کودونی ژمارە 31 و ئەوی تریان له ناوچەی 3'UTR دایە. چەندین توێژینەوەی ئیپیدیمیولوجیك له سەر پیوەندی نیوان ئەم چەند شیوەیانە و مەترسی چەندین جوری شیرپنجە ئەنجام دراوە. ئەنجامی ئەو توێژنەوانە وای له ئیمە کرد کەوا ئەم توێژینەوە له سەرپیوە ندی نیوان چە ند شیوەیی جینیp21 و ئەگەری توش بوونی شیرپەنجەی پروستات له نیو بەشیك له دانیشتووانی پاریزگای کوردستانی ئیران دا ریك خەین. بو دیاری کردنی جینوتایپەکان لە تەکنیکی PCR-RFLP کەلك وەرگیرا. کومەلەی تاقیکراو بریتی بوون له 45 نمونەی بایوپسی وەرگیراو له تووشبووانی شیرپەنجەی پروستات و 45 نمونەی خوینی وەرگیراو له مروفە ئاساییەکان. ئاکامی تەکنیکی PCR-RFLP له کودونی31، بو ئالیلیC، 169جووت باز بوو و بو ئالیلی A، 115 جووت باز و 54 جووت باز بوو. هەر وەها له ناوچەیی3'UTR دا ئاکامی تەکنیکی PCR-RFLP ، بو ئالیلیC، 76 جووت باز بوو و بو ئالیلی T، 115 جووت باز بوو. ەەروەها فراوانی جینوتایژیەکانی C/C و C/A کوی A/A ، له کودونی 31 دا بە ریژەی دەرکەوتنیان بریتی بوون له 31 (٪ 68/8) و 14(٪31/2) بو تووش بووانی شیرپەنجە و 18(٪60) و 12(٪40) بو مروفە ئاساییەکان. فراوانی ئالیلیەکان له نیوان مروفە ئاساییەکان بو ئالیلی C، 0/866 بو، هەر وەها بو ئالیلی A 0/124 بو. بەلام بو تووش بووانی شیرپنجەکە بو ئالیلی C، 0/84 و بو ئالیلی A، 0/16 بوو. بو ناوچەی تە نیا جینوتایپی وەبەرچاو ەاتوو C/C بوو بو مروفە ئاساییەکان و بە نەخوشەکانیشەوە. هەرچەند ژمارە مروفە تاقیکراوەکان زور نەبوو، بەلام ئەو دوزینەوانەی سەرەوە وا دەردەخەن چە ند شیوەیی جینیp21 دەتوانی جوریك له پیوەندی بە تووشبونی شیرپەنجەی پروستاتەوە هەبی.

الخلاصة

العلاقة بين أشكال البروتين P21 المتعددة وسرطان البروستات في محافظة كوردستان, أيران.

P21 هو بروتين CDKI ويؤدي دوراً محورياً في وقف نمو الخلايا وفي التمايز النهائي للخلايا وكذلك في موت الخلايا المبرمج. هناك شكلين او نوعين من P21 وهما كود 31 و3'UTR. وقد اجريت عدة دراسات لغرض بيان العلاقة بين الشكلين اعلاة و ألأصابة بعدة أنواع من السرطان. وهذا كان سبباً دفعنا الى ألقيام بدراسة العلاقة بين الشكلين اعلاه وسرطان البروستات على عينة من سكان محافظة كوردستان في أيران. وشملت الدراسة (45) شخصاً من المصابين بالسرطان وقورنت النتائج مع عينة عشوائية من (45) شخصاً آخرين.

وقد تم التنميط الجيني بواسطة RFLP–PCR باستخدام DNA. وكانت المنتجات RFLP–PCR bp169 للأليل C، وbp115 bp54 للأليل A في codone 31 و تم التحقيق في BP 191 للأليل C، وbp76 bp115 للأليل T في 3'UTR. ترددات C / C و C / A زائد A / A الموروثات في codone 31، كانت 31 (68.8٪)، و 14 (31.2٪) في حالات سرطان و 18 (60.0٪)، و 12 (40.0٪) في حالات السيطرة على التوالي . كانت التوزيعات أليلية لعناصر 0،866 0،124 لC ولـA وكانت للمرضى 0،84 و 0.16 على التوالي لC وA. لUTR'3 كانت الوراثي لاحظ فقط C / C لكل من المجموعتين. على الرغم من أن حجم العينة صغير نسبيا، الى أن النتائج أظهرت أحتمالية وجود العلاقة بين الشكلين أعلاه والإصابة بسرطان البروستات.

ANTI-MULLERIAN HORMONE IS A SIGNIFICANT MARKER FOR MALE INFERTILITY

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ABSTRACT

Background & objective: Anti-Mullerian hormone is a glycoprotein that belongs to the transforming growth factor- B(TGF-B) a member of the super family of growth & differentiation factors. It is thought to be involved in the inhibition of steroid hormone production in women of reproductive age. We aimed to evaluate serum anti-Mullerian hormone (AMH) in men with normal, reduced sperm concentration and in azoospermic men as a possible clinical marker of male factor infertility.
Design Prospective study
SettingPrivate clinic of Dr. Yasir Al-Wattar and Al-Batool Teaching Hospital in Mosul.
Subjects & Methods: This study was conducted on one hundred male subjects aged less than 50 years for the period from July2011-November2011, infertile men were classified according to their sperm count into oligospermics (n=28) and azoospermics(n=47), twenty five men were normal fertile (n=25).Serum concentrations of FSH and Testosterone were measured using ELISA,serum Anti-Mullerian hormone concentrations were measured using AMH/MIS enzyme linked immunosorbant assay kit.
Results: Significant differences in serum Anti- Mullerian hormone, follicle stimulating hormone and Testosterone concentrations were found between normal, oligospermic & azoospermic men.Anti-Mullerian hormone negatively correlated with follicle stimulating hormone in oligospermic and azoospermic men and positively with testosterone in azoospermics and negatively with testosterone in oligospermics.
Conclusion: Anti-Mullerian hormone may serve as a marker of male infertility.

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Key words: Anti-Mullerian hormone, male fertility, follicle stimulating hormone, azoospermia.

Anti-Mullerian hormone (AMH) is a glycoprotein that belongs to the transforming growth factor- B (TGF-B) a member of the super family of growth and differentiation factors. It was identified as a factor which is being synthesized by testicular Sertoli cells, induces regression of the Mullerian ducts during male fetal development. [1,2] It continues to produced by the testes throughout life. It is thought to be involved in the inhibition of steroid hormone production in women through the reproductive age. [3]

AMH not only induces regression of the Mullerian ducts during male sexual differentiation [4-6] but also plays a critical paracrine role in the regulation of Leydig cell development and testosterone biosynthesis. [7,8]

AMH has also been shown to inhibit proliferation of prepubertal progenitor Leydig cells and prevent regeneration of Leydig cells after chemical ablation. [9]

In the male, AMH level rises rapidly after birth, is highest during late infancy, then gradually declines until puberty. [10]

The decrease of AMH corresponds to the Sertoli cell maturation status with the onset of puberty and spermatogenesis, AMH is expressed only by immature Sertoli cells and by immunohistochemistry found only in tubules with spermatogenic arrest or Sertoli-cell-only syndrome. [11,12]

The aim of the study is to verify in well-

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characterized men with normal or decreased sperm count, whether serum AMH determination would add any diagnostic advantage over current endocrine diagnostics.

METHODS

This prospective study was conducted on one hundred men aged less than 50 years who attended an infertility clinic of Dr. Yasir Al-Wattar and infertility clinic at Al-Batool Teaching Hospital between July2011- November2011.

Infertile men were grouped into azoospermic men (n=47) their mean age 35.87±7.32 years and oligospermic men (n=28) their mean age 38.25±8.78years.

Twenty five normal men their mean age 29.96±4.34 years were used as control.

All subjects were questioned about IVF trials, testicular biopsy , mumps , venereal disease , varicocele, drugs which may interfere with fertility and all underwent complete clinical and physical examination.

The subjects considered infertile according to WHO criteria. [13]

Serum concentrations of FSH and testosterone were measured using specific ELISA assay kits.

Serum AMH concentrations were measured using AMH/MIS ELISA kit (immunotest material USA)

Statistics

Data analysis was done using Minitab program. Pearson correlation and unpaired T-test was used. P- value ≤ 0.05 was considered significant throughout the study.

RESULTS

The laboratory values of measurable hormones were significantly different in infertile men as compared to normal men as shown in(Table 1). Serum testosterone and AMH concentrations were significantly lower in oligospermic and azoospermic men when compared to controls. While serum FSH concentrations were found to be significantly higher in patient group than controls.

(Table 2) shows that serum testosterone and serum AMH concentrations were significantly lower in azoospermic men as compared to normal men. While serum FSH was significantly higher in azoospermic men as compared to control men.

(Table 3) shows that oligospermic men had higher testosterone and AMH and lower serum FSH concentrations as compared to azoospermics.

AMH correlated negatively with FSH and testosterone in oligospermic men (Table 4) while in azoospermic men there was a positive correlation between serum AMH and testosterone and a negative correlation between serum AMH and FSH concentrations were found.

Table 1. Mean±SD of measurable serum hormones in infertile men and controls.

Hormones	Infertile men(n=75)	Control(n=25)	Unpaired t-test t-value	p- value
Testosterone(ng/ml)	4.24±2.12	6.48±1.62	5.52	***
FSH mIU/L	17.5±14.9	5.31±1.56	-6.94	***
AMH(ng/ml)	1.30±0.16	3.03±0.48	9.24	***

Table 2. Comparison of measurable serum hormones in azoospermic and control men.

Hormones	Azoospermics (n=47)	Control(n=25)	Unpaired t-test t-value	p-value
Testosterone(ng/ml)	3.33±1.44	6.48±1.62	-8.15	***
FSH mIU/L	24.4±14.9	5.31±1.56	8.70	***
AMH(ng/ml)	0.544±0.80	3.03±0.48	-16.35	***

Table 3. Comparison of measurable serum hormones in azoospermic and oligospermic men.

Hormones	Azoospermics (n=47)	Oligospermics (n=28)	Unpaired t-test t- value	p-value
Testosterone(ng/ml)	3.33±1.44	5.76±2.22	-5.18	***
FSH mIU/L	24.4±14.9	5.05±1.73	8.80	***
AMH(ng/ml)	0.544±0.80	2.58±1.23	-7.79	***

Table 4. Correlation analysis between serums AMH & FSH, serum AMH &testosterone in men grouped according to study protocol.

Hormone	Group	r-value
FSH(mIu/L)	Control men(n=25)	0.282*
	Oligospermic men(n=28)	-0.232**
	Azoospermic men(n=47)	-0.42**
Testosterone(ng/ml)	Control men(n=25)	0.089*
	Oligospermic men(n=28)	-0.161*
	Azoospermic men(n=47)	o.376**

** = non significant*
*** = significant (p-value≤0.05)*

DISCUSSION

Several studies with questionable results have focused on the value of serum FSH and AMH to predict the status of spermatogenesis in the testes of men.

The present study compares some serum biomarkers in normospermic , oligospermic and azoospermic men. The results showed significant difference in FSH, testosterone and AMH concentrations in azoospermic men as compared to normospermic men.this is an indication of defective spermatogenesis and as a result of feedback control probably by inhibin B which is a member of the transforming growth factor (TGF)-β superfamily or may be a direct involvement of AMH. [14]

Serum AMH was found to be significantly lower in men with oligospermia and azoospermia as compared with controls. This is in accordance with results of a previous study, [15] the regulation of AMH after birth is complex; basal levels of AMH are independent of gonadotropin regulation, for example, during childhood and in patients with hypogonadotropic hypogonadism. [16]

AMH was found to be negatively correlated with serum testosterone this in agreement with other studies. [17] , AMH negatively modulates Leydig cell differentiation and testosterone synthesis through its receptors on Leydig cells. [18]

The negative correlation between AMH and FSH this in lines with previous studies,[19,20] either might reflect an involvement in the signaling and regulation of FSH or most probably to be a symptom of impaired or immature Sertoli cells. [21]

In conclusion, AMH should be carefully evaluated in oligospermic and azoospermic men. As anti-Mullerian hormone is a marker of both Sertoli cell proliferation and protein synthesis activity in response to FSH before puberty and also a useful marker of FSH action in the assessment of testicular function in the prepubertal boys. [14]

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پوخته

هورمونێ دژی مولێریان نیشانەکا بەرچاڤە بو نەزوکیا زەلامان

پێشەکی وئارمانج:هورمونێ دژی مولێریان پروتینەکە ژ گروپێ فاکتەرێن وەرارێ کو بێ دیارە رێژا هورمونێن ستیرویدی لدەف ئافرەتێ کێم دکەت. ئارمامجا ڤەکولینێ هەلسەنگاندنا ئاستێ ڤی هورمونێ لدەف زەلامان ئەوێن توخماڤا وان نورمال، کێم و بێن بێ توخماڤ.

شێواز: ڤەکولینەکا دویڤچوونی یە.

ڕێکێن ڤەکولینێ: سەد زەلام بەشداری ڤێ ڤەکولینێ بوون کو ژیێ وان کێمتر بوو ژ 50 سالان دناڤبەرا تیرمەها 2011ێ هەتا چریا دوێ 2011. نەزوکیا ڤان زەلامان هاتبوو گروپکرن لدویف ژمارا توخماڤا دئاڤا وان دا، بێن هیچ نەبن (47 کەس)، بێن کێم هەبن (28) و بێن نورمال (25). هورمونێن رەگەزی هاتنە پیڤان بریکا ELISA وهورمونێ دژی مولێریان بریکا AMH/MIS.

ئەنجام: جیاوازییەکا بەرچاڤ هاتە دیتن یا هورمونێ دژی مولێریان دگەل هورمونێ، رەگەزی دناڤبەرا زەلامێن نورمال و بێ، توخماڤێن وان کێم یان نەی. پەیوەندیا هورمونێ دژی مولێریان یا نەرینی بوو دگەل هورمونێ FSH لدەف زەلامێن نەزوک و یا ئەرینی بوو دگەل هورمونێ تێستوستیرون.

دەرئەنجام:هورمونێ دژی مولێریان دبیت بهێتە بکارئینان وەک نیشاندەرەک بو نەزوکیا زەلامان.

الخلاصة

هرموناتنى مولي رين كعلامة فارقة لعقم الرجال

مقدمة:أجريت الدراسة على مئة رجل معدل أعمارهم اقل من خمسين سنة للفترة من تموز 2011–تشرين الثاني 2011 وذلك لتقييم تركيز هرمون أنتي مولي رين في الرجال الطبيعيين والرجال اللذين لديهم قلة في تركيز النطف واللذين لديهم انعدام النطف ولمعرفة إذا كان من الممكن استخدامه كعلامة في عقم الرجال.

طرق البحث: تم تصنيف الرجال العقيمين حسب عدد النطف الى مجموعة قلة النطف والى مجموعة انعدام النطف كذلك شملت الدراسة خمسة وعشرون رجل طبيعي.

تم قياس مستوى هرمون أنتي مولي رين وهرمون المحفز للجريب والهرمون الذكري بواسطة جهاز أل ELISA.

النتائج:أظهرت النتائج اختلاف واضح في تركيز هرمون إلانتي مولي رين , الهرمون المحفز للجريب والهرمون الذكري عندما تمت مقارنتهم بين الرجال الطبيعيين والرجال العقيمين .

ألأستنتاجات:كذلك لوحظ وجود علاقة عكسية بين هرمون أنتي مولي رين والهرمون المحفز للجريب في الرجال العقيمين بينما كان هناك علاقة طردية بين هرمون أنتي مولي رين والهرمون الذكري في مجموعة انعدام النطف بينما كانت العلاقة عكسية مع الهرمون الذكري في مجموعة انعدام النطف.

SOME OF THE PHYSICAL PROPERTIES OF MODIFIED MICROHYBRID COMPOSITE (AN IN VITRO STUDY)

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ABSTRACT

Background and Objectives: The aim of this study was to measure the water sorption (WS), water solubility (SO) and depth of cure (DC) of modified microhybrid composite resins by adding 2 types of nanofillers in two concentration 3% and 5% and comparing them to unmodified microhybrid composite resins and to nanofilled composite resin.

Methods: For water sorption (WS) and solubility (SO), ten disk-shaped specimens of each resin composite were made for each group and stored in desiccators until constant mass was achieved. Specimens were then stored in water for 7 days, and the mass of each specimen was measured. The specimens were dried again and weighed. The WS and SO were calculated from these measurements. For depth of cure test ten metal moulds with 6 mm long and 4 mm in diameter were prepared then filled with the tested materials. After irradiation, the uncured material gently removed, and measured by micrometer. Data analyzed by one-way ANOVA.

Results: The results showed there were significant differences among the groups in the WS mean values which ranged from 10.05 to 20.69 µm/ mm³, while the SO mean values were ranged from 0.2727 to5.8765 µm/ mm³. The results of depth of cure showed significance differences among the groups and mean value ranged from 0.99500 to 1.9325 mm.

Conclusion: The addition of ZnO to microhybrid composite increased the WS and SO values and decreased the depth of cure values compared to the control group, while the addition of CaCO3 to microhybrid composite had no significant effect on the SO and DC values but they significantly increases the WS compared to the control.

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Key words: Resin composite, Sorption, Solubility, Depth of cure

The current trend in modern resin based composites (RBCs) of minimizing filler size whilst aiming to improve the filler loading has sought to optimize the resultant mechano-physical properties and clinical performance. The introduction of so-called 'nanofilled' and 'nano-hybrid' materials therefore appears a logical continuation of this trend [1,2].

Water sorption by composite materials is a diffusion-controlled process, and the water uptake occurs largely in the resin matrix. The water sorbed by the polymer matrix could cause filler – matrix debonding or even hydrolytic degradation of the fillers, and may affect composite materials by reducing their mechanical properties [3,4].

The other physical properties is the depth of cure (DC), it is usually referring to the thickness of a RBC that is "adequately" cured, which is influenced by numerous factors, including the amount, size and type of fillers, RBC shade, photoinitiator type and concentration refractive index mismatch, light irradiation source and irradiation duration [5,6].

Quadrent anterior shine is a microhybrid composite composite resin commonly used for anterior teeth. To use it for posterior teeth, certain modification should be investigated. Accordingly this study was designed to evaluate the physical properties of previously mentioned

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composite such as water sorption, water solubility and depth of cure after the addition of nano-sized fillers of Calcium carbonate and Zinc Oxide in two different concentrations.

Further studies to evaluate the other properties (mechanical and antibacterial properties) are in progress, and will be published as soon they are completed.

METHODS

Materials

Two commercial composite resins {Microhybrid composite (MH) Quadrent anterior shine (as control group), Nanofilled composite resin Filtek Z350 XT (as reference group)} were used in this study. Two types of silane coated nanofillers (Calcium carbonate and Zinc oxide) both were added to the MH composite. The commercial name, composition and manufacturer of all materials used in this study are listed in (Table 1).

LED (Bluephase C5, IvoclarVivadent] at 400 m W/ cm² was used in this study.

Methods

Preparation of the composite resin specimens

A universal microhybrid commercial composite resin was used as control material and blended with the inorganic nanoparticles. A commercial universal nanofilled composite was used as a reference to compare with the nanoparticle-blended experimental composites (7, 8).

Addition of CaCO3 and ZnO nanoparticles

The CaCO3 and ZnO nanoparticles treated with silane coupling agent manually added to microhybrid RBCs, at two different weight ratios: 3% and 5% depending on the sample group. The mixture thoroughly blended by speed mixture device. The resulting paste packed into teflon molds using an oscillator to remove pores, and covered on both sides with a clear glass plate (7, 8, 9).

Groups design:Six groups of samples denoted MH, N, C3, C5, Z3 and Z5 were defined. The nanoparticle type and weight ratio characterizing of each group are shown in (Figure 1). Each of these groups was subjected to some of the physical tests including the: Depth of cure DC, Water sorption WS and solubility SO.

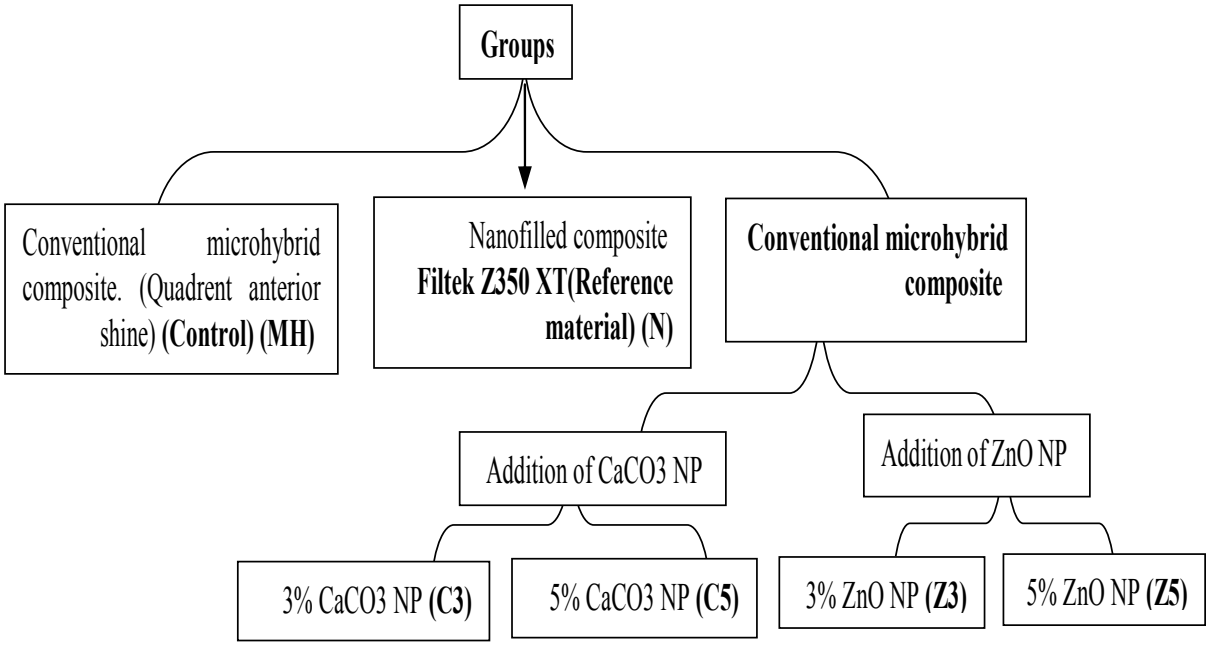

Figure 1. Diagrammatic illustration of experimental design of groups for the study

Evaluation of physical properties:

Depth of cure

Ten metal moulds with 6 mm long and 4 mm in diameter were prepared. The moulds placed on a strip of the transparent film covering the filter paper and fill it with the test material, prepared in accordance with the manufacturer's instructions. The mould and strip of film were pressed between the glass slides to remove excess material. The slide was removed and gently placed the exit window of the external energy source against the strip of film. The material was irradiated for the recommended time to achieve a depth of cure of at least 2 mm. The specimen removed from the mould (180±20) seconds after completion of exposure and the uncured material gently remove with the plastics spatula. The height of the of the cured material was measured with a digital micrometer and the values were divided by 2, this value was recorded as depth of cure. This procedure should be repeated two times as recommended by ANS/ADA Specification No. 27 (10).

2. Water sorption and solubility

Ten mould discs 15±1mm in diameter and 0.5± 0.1mm thick were prepared for each group. The moulds slightly over filled with the material, prepared in accordance with the manufacturer's instructions. Then the exit window of the external energy source placed against the quartz glass plate. The exit window was moved and irradiates a section of the specimen overlapping the previous section of the specimen; the recommended exposure time should be followed in this procedure (10). The cured specimens were removed from the mould and transferred to the desiccators maintain at (37 + 1)°C. After 24 hr the specimens were removed, then stored them in a desiccators maintained at (23± 1°C) for 1 h and then weigh them to an accuracy of ± 0.2 mg. This cycle was repeated until the mass loss of each specimen is not more than 0.2 mg in any 24 h period (m). Then the specimens would be immersed in water and maintain at (37 ±1) °C for 7 days and this mass record as (m2). After this weighing, recondition the specimens to constant mass in the desiccators, the recorded constant mass is (m3). The diameter and the thickness of the specimen would be measured at the center of the specimen and four points on the circumference; calculate the volume, V, in cubic millimeters (10). The values for WS in micrograms per cubic millimeter was calculated using the following equation:

$Wsp = M2-M3/V$

To calculate the values for water solubility (SO) in micrograms per cubic millimeter the following equation was used:

$Ws1 = m – m3/V$

RESULTS

Water sorption (WS) mean values are presented in (Table 2). ANOVA revealed significant differences among the groups ($p<0.05$). MH showed the significantly lowest WS values, followed by C3,C5 and N without statistical difference between them, followed by Z3 were the group with the highest water sorption values.

Water solubility (SO) mean values are presented in (Table 3). ANOVA revealed significant differences among the groups ($p<0.05$). MH, C3, C5 showed the lowest SO values without statistical difference between them, followed by N and Z3 with statistical difference between them followed by Z5 group which showed the highest SO values.

Regarding the depth of cure DC mean values are presented in (Table 4). ANOVA revealed significant differences among the groups ($p<0.05$). Z5 group showed the lowest DC values with statistical difference than the other groups, followed by Z3, followed by C3, C5 and MH without statistical difference between them. The N group showed the highest DC values.

Table 1.The commercial name, the composition and manufacturer of the materials used.

Materials	Composition	Manufacturer
Filtek Z350	Bis-GMA, UDMA, TEGDMA,, Bis-EMA Fillers (78.5%W, 59.5% V): Combination of non-agglomerated/ non- aggregated 20 nm silica filler, non-agglomerated/ non- aggregated 4-11 nm zirconia filler, Aggregated zirconia/silica cluster filler.	3M ESPE, St Paul, MN, USA
Quadrant Anterior Shine	Bis-GMA, acrylates Fillers (75.6% W, 63%V) Barium glass, Silica, silicate glass, fluoride containing fillers (0.7 µm), Polymerization crystal, In-organic pigment	Cavex Holland BV, Haarlem, The Netherlands
Zinc oxide nanofillers	Nanofiller with (30 nm) coated with silane coupling agent (NH2CH2CH2CH2Si(OC2H5)3	Skyspring Nanomaterials, Inc. USA
Calcium carbonate nanofillers	Nanofillers (15 nm) coated with silane coupling agent	M K Impex Corp. Canada

Table 2. Mean values (µg/mm), standard deviations, standard errors and 95% confidence intervals for water sorption WS data.

Groups	N	Mean	SD	SE	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
MH	10	10.05 a	2.01475	0.63712	8.6084	11.4909
N	10	17.04 bc	1.11344	0.35210	16.2443	17.8373
C3	10	16.43 b	1.73010	0.54711	15.1972	17.6724
C5	10	17.3 bc	2.09296	0.66185	15.8005	18.7949
Z3	10	20.69 d	3.95682	1.25126	17.8559	23.5170
Z5	10	18.87 cd	2.23192	.70580	17.2724	20.4656

Different alphabets show significant differences.

Table 3. Mean values (µg/mm), standard deviations, standard errors and 95% confidence intervals for water solubility SO data.

Groups	N	Mean	SD	SE	95% Confidence bInterval for Mean	
					Lower Bound	Upper Bound
MH	10	0.27 a	.02597	.00821	.2542	0.2914
N	10	4.15 b	.81194	.25676	3.5664	4.7281
C3	10	0.55 a	.10202	.03226	0.4716	0.6176
C5	10	0.48 a	.03031	.00959	0.4535	0.4968
Z3	10	4.89 c	1.33200	.42122	3.9406	5.8464
Z5	10	5.88 d	.71864	.22725	5.3624	6.3906

Different alphabets show significant differences.

Table 4. Mean values (mm), standard deviations, standard errors and 95% confidence intervals for depth of cure data.

Treatments	No.	Means	SD	SE	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
MH	10	1.613 c	0.05488	0.01736	1.5737	1.6523
N	10	1.9325 d	0.05780	0.01828	1.8912	1.9738
C3	10	1.622 c	0.09271	0.02932	1.5557	1.6883
C5	10	1.614 c	0.04600	0.01454	1.5811	1.6469
Z3	10	1.1935 b	0.12461	0.03941	1.1044	1.2826
Z5	10	0.995 a	0.07619	0.02409	0.9405	1.0495

DISCUSSION

The amount of water that composite resins can absorb depends on the hydrophilicity of polymeric matrices and filler composition. The groups of composites tested in this study showed non significant different WS mean values after one week of water storage and these values can be considered as lower and adequate for resin-based filler materials. The WS values ranged from 10.05 to 20.69µm/ mm³ and were lower than those required by the ISO 4049 standard, which establishes that the maximum WS value is 40 µm/mm³ [11-13].

The two commercial composites resin (Filtek Z350XT and Quadrant anterior shine) contain BisGMA, other constituent such as di- and methacrylate monomers (Bis-EMA, TEGDMA and UDMA) are also present in Filtek Z350XT, and this difference in the resin matrix may influence on the values for the WS of the tested samples.

It is obvious that the chemistry of the monomers present in the matrix is a key factor to the hydrophilic nature of the polymer [12]. The high viscosity of the polymer requires the addition of diluents monomers, such as TEG-DMA. Such diluents monomers, coupled with the presence of hydroxyl groups in the Bis-GMA molecule, result in an increase in WS. This is in agreement with the result of this study in which nanofilled composite showed higher WS value than microhybrid composite.

It was stated that there is a correlation between SO and degree of conversion in nanofilled and hybrid composites [14]. However, the nanofilled composite presented a higher SO than the hybrid resin. The water SO mean values for the tested groups varied from 0.27 to 5.88 µm/mm³; these values were lower than the maximum value established by the ISO 4049 standard (<7.5 µm/mm³).

Besides unreacted monomers, inorganic ions present as fillers within composites can leach out. In addition, water in contact with silica filler surfaces can break siloxane bonds and the hydrolysis induces debonding of the filler particles, increasing the mass loss of the composites [15]. This may explain why the values for the modified composites groups showed higher WS and SO, particularly the Z3 and Z5 groups.

In the present study, depth of cure was evaluated according to ISO standard scrap test. The same resin composite shade was selected (A3) in order to reduce the possible effect of colorant on photopolymerization. According to the results of this study, Filtek Z350XT exhibited higher DC than other groups of composite resin due to the fact that nanofillers enhance the physical properties of composite resin. The high concentration of a low molecular weight component, TEGDMA in Filtek Z350XT resulted in a system that offered the following advantage: The resultant high number of double bonds per unit of weight on a flexible backbone afforded a high conversion of double bonds during polymerization and increase the DC.

When the CaCO3 was added into the microhybrid composite there was no effect on the DC as demonstrated in the obtained result, while the addition of ZnO nanofillers to the microhybrid composite gave lower DC mean values which were statistically differ than the other groups. It is clear that the size of CaCO3 are less than that of the ZnO, as shown in the (Table 1), and this may lead to occupy substantially greater number of sites in the polymer matrix. The significant increase in specific surface area of filler particles contributes to the fact that CaCO3 had no effect on the DC of the composite resin, in contrary to that the ZnO significantly decreased the DC. The Z5 group showed the lowest DC mean value than Z3 due to the fact that a higher nanofiller content may results in a poor interfacial interaction between matrix and fillers.

CONCLUSION

Filler particles in composite resin seem to have influence upon WS and SO value. The addition of ZnO to microhybrid composite lead to increase the WS and SO values (Although the obtained values are within the acceptable normal limit of ANS/ADA) but it decreases the DC values when compared to the control and reference groups, on the other hand the addition of CaCO3 to microhybrid composite had no significant effect on the SO and DC values but they significantly increased the WS compared to the control and reference groups.

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پوختە

توێژینەوەك دەربارەی چەند سیفەتێكی فیزیایی ماددەی سپی پركردنەوەی ددان

پاش زیادكردنی چەند پێكهاتەیەك

ئامانج: ئامانجی ئەم توێژینەوە یە بریتی یە لە دیاری كردنی رادەی توانەوەی ئاو و مژینی ئاو و پێوەری رەق بوونی ماددەی سپی پركردنەوەی ددان پاش زیادبوونی دوو جۆر لە ماددەی نانۆ بە دوو رێژە 3٪ و 5٪ پاشان بەراووكردنی لەگەل ماددەی پركردنەوەی ددانی سەرەكی و ماددەی نانۆی پركردنەوەی ددان.

رێگای توێژینەوەكە: بۆ توانەوەی ئاو و مژینی ئاو, 10 دیسكی لە ماددەی سپی پركردنەوەی ددان (زیادكراو) ئامادەدەكرێت وهەڵدەگیرێت لە ناو دەفرێكی ووشك كەرەوە تا كێشی دیسكە كە جێگر دەبێت, لە دوایدا دیسكەكان هەڵدەگیرێت لە ناو ئاو بۆ ماوەی 7 رۆژ, پاشان كێشی دیسكەكان دیاری دەكرێتەوە و دیسان كێش دەكرێت.

بۆ دیاری كردنی رادەی رەق بوون و قوولای رەق بوونی ماددەكە, 10 نموومەی ماددەكە ئامادە دەكرێت كەوا 6ملیمتر درێژ بێت و 4ملیمتر پان بێت, دوایی رەق بوونی ماددەكە بە هۆی لایتی (LED), ماددە رەق بووەكە بە هێواش لادەدرێت و بە ئامێری مایكرۆمیتەر دەپێورێت. داتاكە بە رێگەی ANOVA ئاماری لێك دەدرێتەوە.

ئەنجامەكە: ئەنجامی توێژینەوەكە دەركەوت كە وا جیاوازیەكی تەواو هەیە لە نێوان گروپەكاندا.

الخلاصة

دراسة عن بعض الخواص الفيزياوية للمركب الراتنجي المحوّر

الهدف: الهدف من هذه الدراسة كانت قياس الامتصاص والذوبان المائي وسمك التصلب للمركب الراتنجي المحوّر بعد اضافة نوعين من الحبيبات المتناهية الصغر لكاربونات الكالسيوم و اوكسيد الزنك بتركيزين (3٪ و 5٪) ومقارنته مع المركب الراتنجي الغيرمحوّر.

الطريقة: لقياس الامتصاص والذوبان المائي للمادة تم استعمال عشرة أقراص لكل مجموعة وحفظت في جهاز التجفيف (Dessicator) لحين الحصول على وزن ثابت, بعدها تم خزن العينات في الماء لمدة سبعة أيام و قياس الوزن لكل عينة. بعدها تم تجفيف الأقراص وقياس الوزن النهائي. لقياس سمك التصلب تم تحضير عشرة عينات لكل مجموعة من خلال ملئ اسطوانة معدنية, بأبعاد 6ملم طول و4ملم قطر, بالمواد وبعدها تم تعريضها لضوء التصلب, تم استبعاد المركب الراتنجي الغير متصلب, وتم قياس الجزء المتصلب بالمسماك المصغّر (Micrometer).

النتائج: أظهرت النتائج ان هناك فرق واضح بين المجاميع في قيمة معدلات الامتصاص والذوبان المائي وسمك التصلب.

الاستنتاجات: ان الحبيبات المتناهية الصغر لكاربونات الكالسيوم و اوكسيد الزنك لها أثرت على قيّم الامتصاص والذوبان المائي. ان اضافة حبيبات اوكسيد الزنك للمركب الراتنجي يزيد من قيمة الامتصاص والذوبان المائي ويقّلل من قيمة سمك التصلب بالمقارنة مع المركب الراتنجي الغير المحوّر ولكن اضافة حبيبات الكاربونات الكالسيوم ليس لها أي تأثير واضح على قيّم الذوبان المائي وسمك التصلب ولكنها تزيد من قيمة الامتصاص المائي بالمقارنة مع المركب الراتنجي الغير المحوّر.

STUDY OF SERUM MAGNESIUM AND CALCIUM IN PREECLAMPSIA AND NORMAL PREGNANCY

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ABSTRACT

Background and objectives: The cause of pre-eclampsia remains unknown; calcium and magnesium have been blamed to play a role in its pathtogensis. The objective of this study was to assess variations in serum levels of those cations in healthy pregnant women and those with pre-eclampsia.
Methods: This prospective study evaluated the serum levels of magnesium and calcium in the second half of pregnancy in 30 healthy normotensive pregnant women, 30 pre-eclamptic cases, thirty five age - matched healthy non-pregnant women served as a control. The study was conducted in the period of July 2010 - May 2011
Results: Pre-eclamptic women had a significant decrease in mean serum magnesium concentration (1.53 ± 0.65 mg/dL) compared with the healthy normotensive pregnant women (2.26 ± 0.73 mg/dL) and the healthy non-pregnant controls (2.31± 0.29 mg/dL). Pre-eclamptic women had a decrease in mean serum calcium concentration (8.72±0.59 mg/dL) compared with the healthy normotensive pregnant women (8.97 ± 0.52 mg/dL) and the healthy non-pregnant controls (9.12 ± 0.31 mg/dL) but these differences were not significant.
Conclusion: Pre-eclampsia was clearly associated with significant hypomagnesaemia. This association may be significant in understanding the pathophysiological processes of pre-eclampsia. However, the association was not so clear for serum calcium.

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Key words: Pre-eclampsia, hypomagnesaemia, hypocalcemia

Pre-eclampsia is defined as the triad of hypertension, proteinuria occurring after 20 weeks gestation in a previously normo-tensive woman. It is specific to human pregnancies and complicates 6 – 8 % of pregnancies after week 20. Preeclampsia is still one of the leading causes of maternal and fetal morbidity and mortality. Despite intensive research for many years; the etiology of this disorder remains,unknown[1-3].

Magnesium is the 4th most abundant cation in the body and is present in more than 300 enzymatic systems where it is crucial for ATP metabolism[4,5] .Magnesium also plays an important role in neurochemical transmission and peripheral vasodilatation and may influence blood pressure by modulating vascular tone and structure through its effects on a myriad of biochemical reactions that control vascular contraction/dilation. Magnesium acts as a calcium channel antagonist. It stimulates the production of vasodilator prostacyclins and nitric oxide and alters vascular responses to vasoconstrictor agents. Its deficiency can also play a role in hypertension of pregnancy[6-8]. Magnesium and calcium have been implicated in seizure disorders, which often complicate preeclampsia[9-12]. Magnesium sulfate, as it appears to be safe and effective for the prevention of seizures, has been used as the drug of choice in severe pre-eclampsia and eclampsia treatment[11,12]. It is imperative to evaluate the levels of this cation in our pregnant ladies. Calcium plays an important role in muscle contraction and regulation of the water balance in cells. Modification of plasma calcium concentration leads to the alteration of blood pressure. The lowering of serum calcium and the increase of intracellular calcium can cause an elevation of blood pressure in pre-

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eclamptic,mothers[13-16].
There are several studies in this field, while some studies support the hypothesis that low serum magnesium and or calcium play an important role in the pathophysiology of systemic arterial hypertension and in the etiology of pre-eclampsia; other studies do not support this notion.
The objective of this study was to assess serum magnesium and calcium levels in healthy normotensive pregnant women in order to compare them with those in pre-eclamptic cases with the aim of providing preliminary data that could be useful in the management of pre-eclampsia and eclampsia.

METHODS

This cross-sectional study was conducted in Kurdistan region, Erbil maternity teaching hospital between July 2010 and May2011.
The participants were aged 21-33 years and divided into three groups:
First group (G1) comprised 35 healthy non-pregnant women who served as controls.
Second group (G2) comprised 30 normotensive primigravida, > 28 weeks of gestation.
Third group (G3) consisted 30 pre-eclamptic primigravida, > 28 weeks of gestation. Normotensive non-pregnant and normotensive pregnant women were carefully matched for age with the pre-eclamptic,group.
Pre-eclampsia was defined as development of blood pressure >140/90 mm Hg after the second half of gestation and proteinuria of at least 1+ on dipstick testing with or without abnormal heamogram, renal, liver or-coagulation-tests.
Patients with a history of medical diseases such as hypertension, renal disease, diabetes mellitus, heart failure, ischaemic heart diseases, endocrine abnormalities and patients on magnesium sulphate and or calcium lactate medications were excluded from the study. All participants gave informed consent after due explanation before enrollment in .All participants gave informed consent after due explanation before enrollment in the study. Blood samples were collected from the anticubital vein, centrifuged at 5,000 rpm for 5 minutes. The serum was analyzed for magnesium and calcium using bt 35i auto - analyzer.
The blood sample was also sent for complete blood count, prothrombin time, partial thromboplastin time, sugar, renal and liver function tests.

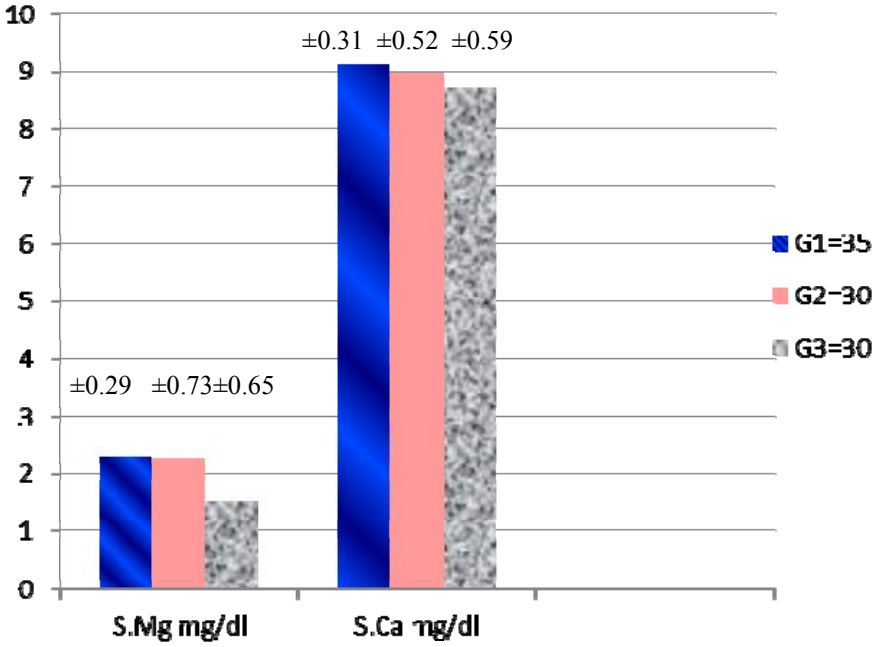
Statistical Analysis

The values of the laboratory parameters are presented as the Mean±SD. A Student's unpaired t-test was used for cross sectional comparisons of variables between the groups. The results were considered statistically significant when the probability of the null hypothesis in this study was less than 5% ($p<0.05$).

RESULTS

The mean age of the participants was matched in the three groups (26±8.1, 27±3.4, and 24±9.8 years) respectively.
The mean systolic blood pressure (MSBP) was 115.6 ± 10.1 in healthy non-pregnant group G1, 121.3± 8.4 mmHg for normotensive pregnant women G2 and 169.4 ± 22.1 mmHg for pre-eclamptic pregnant women G3.
The mean diastolic blood pressure (MDBP) for the three groups was 72.1 ± 12.5 in G1 mmHg, 69.3± 9.2 mmHg in G2 and 109.3 ± 11.8 mmHg in G3.
There was no significant difference in serum magnesium level between healthy non-pregnant women G1 (2.31± 0.29 mg/dL) and normotensive pregnant women G2 (2.26 ± 0.73 mg/dL), but the serum magnesium level was significantly higher in these 2 groups as compared to the pre-eclamptic pregnant women (1.53 ± 0.65 mg/dL).
Although the plasma calcium was higher

in the healthy non-pregnant women G1 (9.12 ± 0.31 mg/dL) than in the normotensive pregnant women G2 (8.97 ± 0.52 mg/dL) and in the pre-eclamptic pregnant women G3 (8.72±0.59 mg/dL) but these results were not of statistical significance and all in the normal range of serum calcium level.



Comparisons of the serum magnisium & calcium for the three groups

	G1 (n = 35)	G2 (n = 30)	G3 (n = 30)
Age (years)	26±8.1	27±3.4	24±9.8
M.S.B.P (mmHg)	115.6 ± 10.1	121.3± 8.4	169.4 ± 22.1
M.D.B.P (mmHg)	72.1 ± 12.5	69.3± 9.2	109.3 ± 11.8
S.Mg+2 (mg/dL)	2.31± 0.29	2.26 ± 0.73	1.53 ± 0.65 *Ω
S.Ca+2 (mg/dL)	9.12 ± 0.31	8.97 ± 0.52	8.72±0.59s

Values are given as mean + SD; n: number of participants
M.S.B.P : mean systolic blood pressure
M.D.B.P mean diastolic blood pressure
** P < 0.05 pre-eclamptic compared with non-pregnant women*
Ω P < 0.05 pre-eclamptic compared with normotensive pregnant women

DISCUSSION

Calcium and magnesium are two intracellular ions that are very important for cellular metabolism such as muscle contractility, secretions, neuronal activity as well as cellular death. The immunomodulatory properties of magnesiu calcium1, 25-dihydroxyvitamin D3 may be relevant in this regard.

In the present study, we were unable to find any significant differences in serum magnesium levels in normotensive pregnant women compared with healthy non-pregnant women but we found decreased serum magnesium levels in pre-eclamptic women compared to both normotensive pregnant women and healthy non-pregnant women. This is compatible with other studies [17-20]. In other studies, however, there was no significant difference in serum magnesium among normal pregnancy and pre-eclampsia[21-23]. The serum magnesium in our study decreased significantly and this reduction may be one of the important factors

responsible for the pathophysiological changes of pre-eclampsia. This support Kesteloot `s hypothesis that the hemodilution effect of oestrogen and increased demand of the fetus in normal pregnancy slightly decreases the serum magnesium level and in pre-eclampsia there will be further decreases in the serum magnesium level due to increased urinary excretion of magnesium[24]. Magnesium deficiency causes hemodynamic abnormalities such as arterial wall thickening, abnormal vascular tone and endothelial dysfunction which are due to alteration in the biology of cellular and non cellular components of the arterial wall.This may be an important aetiological factor and explain causal relationship between hypomagnesaemia and pre-eclampsia since magnesium is involved in blood pressure regulation through an intracellular inhibition of NO synthase in endothelialcells[25],Hypomagnesemia is associated with impaired production of ATP and ATP dependent sodium / potassium and calcium pump, providing another hypothesis to explain the association of hypomagnesemia with pre-eclampsia[26].

In the present study, the variables of serum calcium showed some reduction from the first group down to the third one but these changes were not statistically significant. Ingec[27],Szmidt-Adjide[28]and ahidrodsari,[29] ; supported, but Lopez-Jaramillo[30]and others[31-33] didn't support this finding; as there have been conflicting results among those studies raising doubts about the exact role of calcium in the pathophysiology of pre-eclampsia. Those studies who revealed a significant reduction in serum calcium level in pre-eclampsia supported the hypothesis that calcium might be a cause in the development of preeclampsia and stated that the effect of serum calcium on changes in the blood pressure could be explained by the level of intracellular concentration of calcium as the increase of intracellular calcium concentration when the serum calcium decreased led to constriction of smooth muscles in the blood vessels and increase of vascular resistance.

They claimed that hypocalcemia related to maternal vitamin D deficiency may predispose to the increased inflammatory response. Notably, Vitamin D deficiency may also elevate blood pressure as vascular structure and function including vascular compliance, elasticity and intima media thickness is less favorable among those women.As vitamin D plays an important role in calcium metabolism; it had been found that maternal vitamin D deficiency at less than 22 wk gestation was a strong, independent risk factor for preeclampsia[34]. Importantly, there was a monotonic dose-response relation between maternal serum 25(OH) D and risk of preeclampsia[35].

Haugen et al36 claimed that there will be 27% reduction in risk of pre-eclampsia for women on vitamin D intake 10-15 microg/day with /out 1000 mg/day calcium.

Studies of seasonal patterns in preeclampsia showed the lowest incidence in summer, when sunlight is plentiful and serum 25(OH) D concentrations are at their peak, and the highest incidence in winter, when synthesis of vitamin D3 is limited in temperate zones and serum 25(OH) D levels are at their nadir[37]. However, our present finding showed that the mean serum calcium level in pre-eclampsia was not significantly different from healthy non-pregnant and normal pregnant-participants.

These contradictory outcomes may be explained by the variations of the studied population and the difference in daily dietary intake of calcium among different populations.

CONCLUSION

This study clearly revealed a significant reduction of serum magnisium in pre-eclamptic cases supporting the important contribution of magnisium in the

pathophysiology of pre-eclampsia and explaining the importance of magnisium in the management. The difference in serum calcium was not significant, adding further dilemma to the role of calcium in the pathogenesis of pre-eclampsia, necessitating further studies to investigate such important issue.

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پوختە

هەڵسانگندنی مەسلی مەگنیسیۆم و کالسیۆم دەکات لە نیوان سکپڕی ئاسایی و پێش ژەهر بوونی سکپری

ئامانج و شارەزایی ڕابردوو: تا ئێستا ژەهراوی بوونی ئافرەتی سکپری نادیارە ، و ە هۆیەکەشی دەگەڕێتەوە بۆ کالسیۆم و مەگنیسیۆم کە هەردوکیان ڕۆلێکی سەرەکیان هەیە لە pathtogensis. ئامانج لەم لێکۆڵینەوەیە بۆ هەلسەنگاندنی گۆرانکاری لە ئاستی مەسلی خوین لەناو ئەو کاتیۆناتانەیە کە لە ئافرەتی سکپری تەندروستی باش هەیە لەگەڵ ئەوانەی پێش ژەهر بوونی سکپری دەبن.

نەخۆشەکان و ڕێگاکانیان: ئەم لێکۆڵینەوەیە هەڵسانگندنی مەسلی مەگنیسیۆم و کالسیۆم دەکات لە نیوەی دووەم ماوەی سکپڕی بۆ 30 ئافرەتی سکپڕ تەندورستی باش لەگەڵ ئەوانەی کە زەختی خوێنیان ئاساییە و 30 ئافرەتیش لە حالەتی پێش ژەهر بوونی سکپری وەئەم لەگەل 35 حالەتیش لە و ئافرەتانەی کە سکپڕ نین و تەندروستیان باشە کە هاوشێوەن لە تەمەندا وەک گروپی بەراوورد . ئەم لێکۆڵینەوەیە لەم ماوەیە جێبەجێکراوە لە مانگی حوزەیرانی 2010 تا مانگی ئایاری 2011.

دەرەنجامەکان: ئەو ئافرەتانەی کە تووشی پێش ژەهربوونی سکپڕی بوون کەمی خەستی مەگنیسیۆم زۆر بە ئاشکرا لە خویندا دیاربوو (1.53 ± 0.65 mg/dL) بەبەراوورد لەگەڵ ئەو ئافرەتانەی کە سکپڕ بوون بەتەندروستی باش و زەختی خوێنی ئاسایی کەبریتی بوو لە (2.26 ± 0.73 mg/dL) . لەگەڵ ئەو ئافرەتانەی کە سکپڕ نین وەکو گروپی بەراوورد(2.31± 0.29 mg/dL) بۆیە دیترا کەوا ئافرەتی پێش ژەهربوونی سکپڕ کەم بوونەوەیەکی زۆری هەیە لە خەستی کالسیۆم لە ناو خوین (8.72±0.59 mg/dL) بەبەراوورد لەگەڵ ئافرەتی سکپری تەندوستی باش وە زەختی خوینی ئاسایی (8.97 ± 0.52 mg/dL) لەگەڵ ئافرەتی ناسکپڕ بەتەندورستی باش و زەختی خوینی ئاسایی (9.12 ± 0.31 mg/dL) کە کونترۆلی کردوە بەلام ئەم گۆڕانکاریانە زۆر نین.

پووختە: ژەهربوونی پێش سکپری پەیوەندی بە گرنگ و بایەخی hypomagnesaemia باش دیار بوو وەئەم پەیوەندیە لەوانەیە گرنگ بێت بۆ گەیشتنی پرۆسێسەکانی نەخۆشی لە لەشی نەخۆشدا لە ژەهربوونی پێش وەخت لە سکپری بەڵام سەرەڕای ئەمەش ئەم پەیوەندیە ئاشکرا نەبوو لە ئاستی مەسلی کالسیۆم.

الخلاصة

دراسة مستويات المغنيسيوم والكالسيوم في الدم بين النساء الحوامل ألأصحاء والحوامل قبل تسمم الحمل

الخلفية والأهداف: إن سبب تسمم الحمل ما زال مجهولا ، وقد ألقي باللوم على عنصري الكالسيوم والمغنيسيوم و اللذان قد يلعبان دورا في حدوث المرض .كان الهدف من هذه الدراسة هوتقييم التغيرات في مستويات مصل الدم من تلك الكاتيونات في النساء الحوامل الأصحاء ومن المصابات بحالة ما قبل تسمم الحمل.

المرضى والطرق: في هذه الدراسة المستقبلية، تم قياس مستويات المغنيسيوم والكالسيوم في الدم في النصف الثاني من الحمل لـ 30 امرأة حامل مع ضغط الدم الاعتيادي، و 30 حالة ما قبل تسمم الحمل و 35 من النساء الأصحاء غير الحوامل و بأعمار متقاربة كمجموعة مقارنة. وقد أجريت الدراسة في الفترة من حزيران 2010 الى ايار 2011 .

النتائج: إن النساء اللواتي يعانين من حالة قبل تسمم الحمل لديهن انخفاض ملحوظ في متوسط تركيز المغنيسيوم في الدم (0.65±) 1.53ملغ / دسل) بالمقارنة مع النساء الحوامل الأصحاء ذوات ضغط الدم الاعتيادي (2.26 ±0.73 ملغ / دسل) و النساء غير الحوامل الأصحاء من مجموعة المقارنة (2.31 ± 0.29 ملغ / دسل) لوحظ لدى نساء ما قبل تسمم الحمل انخفاضا في متوسط تركيز الكالسيوم في الدم (8.72 ±0.59 ملغ / دسل) بالمقارنة مع النساء الحوامل الأصحاء ذوي ضغط الدم الاعتيادي (8.97±0.52 ملغ / دسل) مع النساء غير الحوامل الأصحاء (9.12 ± 0.31 ملغ / دسل) ولكن هذه الاختلافات ليست كبيرة

الخلاصة: كانت حالة ما قبل تسمم الحمل مرتبطة بشكل واضح بأنخفاض مستوى المغنسيوم في الدم و قد يكون هذا الارتباط الكبيرمهما في فهم العمليات المرضية في حالة ما قبل تسمم الحمل ومع هذا فأن الارتباط لم يكن واضحا بالنسبة لمستوى الكالسيوم .

ARTHROSCOPIC MANAGEMENT OF KNEE DISORDERS IN ERBIL HOSPITALS RETRO-PROSPECTIVE STUDY

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ABSTRACT

Objective: To analyze and evaluate the findings of our twelve months arthroscopy practice starting February 1, 2010 till January 31, 2011 in the department of Orthopedics for patient underwent operation of arthroscopy for management of meniscal lesions within Erbil city hospitals, governmental and private.
Aim of Study: The aim of this study is to evaluate the short term result and to analyze their data according to age, sex distribution, mode of trauma, clinical findings, radiographic x ray and MRI findings, anaesthesia type, duration of hospital stay, performed procedures and arthroscopic findings and their short term clinical and functional outcomes .
Methods: This is a retro - prospective study of 160 patients who underwent knee arthroscopy for meniscal lesions at Erbil city hospitals. Patient ages ranged from 21 to 70 years old with an average age of 39.5 years.
Results: Arthroscopy was performed on 82 right and 78 left symptomatic knees of 113 males and 47 females, the etiology were classified as sport injury in 82 knees (51.25 %), falling 30 knees (18.75 %), traffic accident 17 knees (10.62 %), working accident 8 knees (5%) and un known 23 knees (14.37 %). Arthroscopic findings of patients were posterior horn tear of medial meniscus in 61 knees(38%), anterior horn tear of medial meniscus in 30 knees (18%), anterior and posterior horn tear of medial meniscus in 2 knees (1.25%), bucket handle tear of medial meniscus in 24 knees (15%), Degenerative tear of medial meniscus in 8 knees (5 %), and anterior horn tear of lateral meniscus 14 knees (8.75%) and posterior horn tear of lateral meniscus 3 knees (1.25%), bucket handle tear of lateral meniscus in 1 knee (0.6%) and tears involving both menisci in 6 knees (5.6%) and normal menisci in 11 knees (6.8%).
Meniscal lesions were associated with complete tear of ACL in 12 knees (7.5%), partial tear of ACL in 30 knees (18%), partial tear of PCL in 3 knees (2%), complete tear of PCL in 4 knees (2.5%).
Tight and tethered plica was founded in 19 knees (11%) distributed as medial patellar plica in 13 knees, infrapatellar plica – ligamentum mucosum in 5 knees and suprapatellar plica in 1 knee. Small tiny osteochondral bodies were found in 2 knees and in 1 patient there was cyst in lateral meniscus.
Conclusion: because of many advantages and few complication rate and arthroscopic partial menisectomy to be the definite means of therapy for the isolated meniscal lesion of the knee joint. Quick recovery and excellent early results after surgery are characteristics of the method.
Also arthroscopy is the method of choice in the diagnosis of knee disorders

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Key words: Arthroscopy, Knee disorder

Knee arthroscopy is the most common procedure performed among orthopedic sport surgeons[1]. With the advent of magnetic resonance imaging and other non-invasive techniques, therapeutic arthroscopy comes into practice.
Indications for knee arthroscopy include the treatment of meniscal pathology, specified articular cartilage lesions, costeochondral lesions, loose bodies, advanced synovitis and performing synovial biopsy, cruciate ligament tears and certain tibia plateau fractures [2].
Contraindications for the use of knee arthroscopy are Damage to the posterior capsule of the knee [3] may allow travasation of the fluid into the surrounding tissues. This will lead to swelling and may result in compartment syndrome, joint ankylosis, local skin infection around the potential portal sites which may lead to joint infection sepsis [4].

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The advantages of arthroscopic procedures far out weigh the disadvantages. Among the advantages when compared with arthrotomy are the following: reduced postoperative morbidity; smaller incision; less intense inflammatory response; improved thoroughness of diagnosis; absence of secondary effects; reduced hospital cost[5,6,7]; reduced complication rate which varies from 0.5% with usual procedures as menisectomy and cruciate ligament repair to 2.4% with newer procedure as meniscal repair; improved evaluation at follow-up; possibility of performing surgical procedures that were difficult or impossible to perform through open arthrotomy[8,9,10].

The most common complications are haemarthrosis, infection, thromboembolic disease, complication of anaesthsia, instrument failure, reflex sympathetic dystrophy, ligament injury[11.12.13].

METHODS

This study covers 160 patients underwent arthroscopy operation for management meniscal lesions within Erbil city hospital, during the period from February 1, 2010 till January 31, 2011, data were obtained from the database of the theater, and entered into a proforma sheet, the following points were noted with the history Age, sex, Occupation, Mode of Trauma: (Sport injury, Falling, Traffic Accident, Working Accident, Not Remembered), Joint Involved, Clinical Finding , X Ray Findings, MRI Findings, Type of Anaesthesia, Arthroscopy Findings, Type of Management, and Duration of Stay in Hospital. Routine preoperative investigations and X- ray of the involved joint were done; MRI was done in only 110 patients.

Torn menisci were excised, the aim was to excise the unstable segment of the meniscus and preserve the table and well balanced rim as much as possible. In patients with degenerative joint disease, hypertrophied synovium was shaved. Tight and tethered plicae was found in 19 patient and plica excision was done for them, degenerate menisci were trimmed and osteochondral fragments founded in two patient, they were small and tiny that dose not demands fixation and fragments removal was done for them.

Anterior cruciate ligament reconstruction was done arthroscopically for three patients with anterior cruciate ligament tear.

Portal sites were closed with single suture for each and the knee covered by double layers of cotton and elastic bandages.

All patients instructed and trained to practice physical exercised in the same post operative day in full range of motions concentrating on straight leg – raising and strengthening of quadriceps muscles, weight bearing was encouraged as tolerated with crutches.

The total agreement between the MRI findings and arthroscopic findings were calculated using the positive predictive value PPV and negative predictive value NPV, the PPV is defined as the probability that patient has the disease when restricted to those patient who test positive, and the NPV is defined as patient with negative test that do not have disease.

Inclusion Criteria

All cases with meniscal derangements and pathologies and are undergoing knee arthroscopy within Erbil city hospitals will be included in this analysis. This group includes:

1- Patient with torn meniscus proved clinically or radiologically.

2- Symptoms persisted or recurred after previous meniscal surgery with possibility of retained fragment or a recent tear.

Exclusion Criteria

1- Arthroscopy performed for knee lesions other than meniscus lesions.

RESULTS

The most common age group was 26-35 years, younger age group, because young people are more commonly involved in sports and outdoor activities,
the male to female ratio was 3: I as men are more commonly involved in sports and outdoor activities.
In 30 out of 160 cases, the mode of trauma was fall/ twist. In 23 cases, there was no history of any trauma. 82 patient give history of sports field injury, and 17 cases has meniscal lesion resulted from road traffic accident, and 8 patient had accident in the working field resulted in meniscal tears.
The X-rays was done in all the 160 cases and concluded normal results in 142 patients while osteoarthritic changes were the findings in 18 patients. On M.R.I. findings, out of 160 patients, MRI examination was not conducted in 50 (31, 25%) patients. For the rest of 110 patients for whom MRI examination was conducted and basing on the arthroscopy results as a standard:
32 (29%) Total agreement between MRI reporting and arthroscopic findings, 78 (71%) patient has arthroscopic findings differs from that in their MRI reporting. The positive and negative predictive values were calculated using the following equations;
positive Predictive Value= number of true positive/ number of true positive+ number of false positive
=32/ 32+78= 29%.
Negative Predictive Value = number of true negative/ number of true negative + number of negative values
= 78/ 78+32= 71%.

Table I. Distribution of Cases According to MRI Findings.

MRI Finding	No. of Cases	Percent
MRI Not Arranged	50	31.25%
Anterior Horn Tear Medial Meniscus	3	1.87%
Bucket Handle Tear Medial Meniscus	1	0.6%
Posterior Horn Tear Medial Meniscus	84	52.5%
Normal MRI	20	12.5%
Plica	1	0.6%
Posterior Horn Tear Lateral Meniscus	1	0.6%
Total	160	100%

150 patients had general anesthesia and the rest 10 cases had spinal/ epidural block type of anesthesia.
Ninety eight cases were discharged on the same day. Fifty seven patients were kept for one day, in both groups patients were willing to leave the hospital which reflects no or limited pain in post operative period. One case with ACL reconstruction was kept for one day and four patients with ACL reconstruction were kept for four days in the hospital.
The prospective part of our study had covered 33 patients who had arthroscopic operation in Erbil teaching hospital. Before operation pain was present in all of them of mild to moderate degree, pain associated with giving way specially while climbing stairs was the reason of presentation in 15 patients, pain and locking was in the history of 5 patients, pain and clicking in 8 patients, the rest of 5 patients had history of pain and swelling following strenuous activities. Clinical examination conducted for the 33 patients covering the points of effusion, joint line tenderness, Mc Murrays test (the knee is flexed as much as possible, one hand steadies the joint and the other rotates the leg medially and laterally while the knee is

slowly extended, the test is repeat several times with the stressed in valgus and varus felling and listening the click) and Apleys test (with patient is prone, the knee is flexed to 90 degree and rotated while a compression force is applied, the grinding test reproduces symptom if the meniscus is torn, the clinical findings were distributed as shown in Table below.

Table 2. Distribution of clinical signs.

Clinical Signs	No. of Cases	Percent
Effusion	**12**	**36.36%**
Joint line tenderness	**13**	**39.39%**
Mc Murays test	**4**	**12.12%**
Apleys test	**4**	**12.12%**
Total	**33**	**100%**

For clinical follow up, patients has been called by phone 4-6 weeks after doing the operation, 20 patients were lost to follow up. Patient response to surgery was classified according to their statement, the commonly used statements were (very good, rarely have some pain, same as before surgery and worse than before surgery) according to the criteria's by Tapper and Hoover14.

Patient used statement "very good" classified as having excellent result, patient stated as " rarely have pain" were classified as having good result of surgery, and those stated " same" were classified as to have fair result and finally those stated " deteriorated, worse than before surgery" were classified as poor result. Out of 140 patients 24% gives statement of very good (excellent result), 38% of patients complains of rarely having pain and classified as good result, 20% of patient experienced same complain and symptoms after surgery and classified as having fair result and finally 18% of patient were deteriorated and their knees became worse than before surgery and are classified as having poor result of surgery.

Table 3. Overall results, 4 weeks after arthroscopy.

	No. of Cases	Percent
Excellent	33	24.5%
Good	54	38.5%
Fair	28	20%
Poor	25	18%
Total	140	100%

66% of patients were returned to duty at their original commands, and 34% to limited duty status consisting of no strenuous running, heavy lifting, prolonged walking or prolonged standing.

All patients were instructed to have limited duty with duration averaged 24 days and average duration of post operative hospitalization was 1 day.

Partial Menisectomy was the most commonly performed procedure, in some cases more than one procedure performed in one session. Simple arthroscopic shaving of frayed and fibrillated cartilage with debridement has been attempted after partial menisectomy and for hypertrophied synovium.

We saw 73 knees of concomitant injuries at arthroscopy, the predominant pattern was anterior cruciate ligament tear and medial meniscus tear in 42 patients followed plicae which were founded in 19 patients.

Table 4. Distribution of lesion of meniscus According to Arthroscopic.

Lesion	No. of Cases	Percent
	Medial Meniscus	
Anterior Horn Tear	30	18%
Posterior Horn Tear	61	38%
Anterior & Posterior Tear	2	1.25%
Bucket Handle Tear	24	12.5%
Degenerative Tear	8	5%
	Lateral Meniscus	
Anterior Horn Tear	14	9%
Posterior Horn Tear	3	1.8%
Bucket Handle Tear	1	0.6%
	Combined Medial and Lateral Meniscal lesions	
Posterior Horn Tear of medial and lateral Meniscus	5	3%
Anterior Horn Tear of medial and lateral Meniscus	1	0.6%
	Normal menisci	
Normal Menisci	11	6.8%
Total	160	100%

Table 5 Distribution of Cases According to Arthroscopic findings for concomitant injuries.

Associated lesions	No. of Cases	Percent
Complete ACL Tear	12	7.5%
Partial ACL Tear	30	18.5%
Partial PCL Tear	3	1.8%
Complete PCL Tear	4	2.5%
Plica	19	11.8%
Plica + ACL Partial Tear	2	1.25%
Osteochondral Body	2	1.25%
Cyst in Lateral Meniscus	1	0.6%
None	87	54.3%
Total	160	100%

DISCUSSION

In the past, the meniscus was thought to be a vestigial remnant and therefore an expandable structure[15,16], menisectomy was considered a benign procedure and short term studies showed good results, However, it eventually become obvious that meniscus served a significant function and total menisectomy lead to high percentage of poor clinical results and degenerative radiographic changes[16, 17, 18]. The greatest advantages of arthroscopic menisectomy are; - it's an outpatient department procedure [19], quick rehabilitation, reduced loss of time, low morbidity, and limited approach performed

for suspected meniscal lesions on clinical or radiological basis.

In our series, there were 113 males and 47 females with mean age of 39.5 years ranging from 21-70 years, however maximum number 57 patients (35.6%) were in the 26-35 years, in the study conducted by Russel J.A. Tregonning[20] the mean age was 37 ranging from 15-54 years. In a similar study conducted by Gillquist J21 the range was from 18-78 years and average was 39.7 years.

In the present series, there was 82 right knees and 78 left knees involved, 82 patients (51.25%) gives history of trauma encountered within sports field which is the commonest mode of injury and both studies of Russel J.A. Tregonning and Gillquist [20,21] also had athletics injuries as the commonest causes of meniscal lesions, 23 patient (14.37%) have unspecified or can not remember any kind of reason for the knee problem, 30 patients (18.75%) give history of falling and 17 patients (10.62%) presented with history of road traffic accident. Partial menisectomy was the most commonly performed arthroscopic surgery accounting for 41% of knee joint arthroscopies[22] ,and in our series partial menisectomy account for 86.25% of cases, partial menisectomy was also the performed procedure but with other procedure to manage abnormalities associated with meniscal lesion or described as combined or concomitant lesions which were discovered during the arthroscopic operation as excision of plica, only the tight and tethered one which was performed in 19 patients and ACL repair performed in 3 patients, the plica fold were distributed as: medial patellar plica in 13 knees, infrapatellar plica – ligamentum mucosum in 5 knees and suprapatellar plica in 1 knee.

The medial meniscus was more commonly affected and lesions involving posterior horn of medial meniscus were as high as twice of anterior horn tear of medial meniscus and three times more than bucket handle tear and similar result was also conducted by Verdi V23, tears involving anterior and posterior horn of medial meniscus was found in 2 patients while degenerative tear found in 8 patients.

Furthermore in the degenerative knee, the torn menisci may not be a source of patient symptoms and its removal may not alleviate those symptoms[24], Dandy and Jakson found it's difficult to distinguish between symptom of meniscal and degenerative lesions.

When meniscal tear is associated with ACL tear in young adult, ACL reconstruction is preferable to be done to prevent repeated tear due to instability[25] and in our series only three patients out of twelve patients has ACL reconstruction along with partial menisectomy. And regardless the result of MRI reports, examination of the knee under anaesthesia is important to determine the status of cruciate ligaments or to detect the subtle antero-posterior laxity prior proceeding to arthroscopy. The indication for arthroscopic cruciate ligament repair includes:

The locked knee, pseudo locking.

The professional and highly competitive athlete.

Where physiotherapy had not helped in advancing knee joint rehabilitation.

Our study revealed that MRI reporting system was not reliable to pick up specific meniscal lesion and the total agreement between MRI and arthroscopy was 29% of patients, this matches with other published literature like that by Friemert et al[26], which stated that MRI can not replace arthroscopy for diagnosis of cartilage damage and hence arthroscopy still has to be seen as the method of choice as the sensitivity of arthroscopic diagnosis is relatively high, Kocher et al[27] mentioned that selective MRI dose not provide an enhanced diagnostic utility over arthroscopy or clinical examination and MRI should be preserved for cases where clinical diagnosis is uncertain and when

the input of MRI is likely to alter the treatment plan.

Its not recommend depend on the routine MRI reporting system in our locality to confirm diagnosis as the positive predictive value of the scan is low (29%) taking also into consideration the expensiveness of diagnostic investigation , in the presence of positive clinical signs, we would recommend proceeding to arthroscopy. And in 50 patients MRI was not conducted mostly due to economic load of the MRI. And the lack of expertise and deficiency of professional staff that are familial in reading the MRI films is behind this draw back.

The effectiveness of MRI in diagnosis of meniscal tear can be promoted by using the 1.5 Tesla MRI.

All patients were instructed to have limited duty with duration averaged 24 days and average duration of post operative hospitalization was 1 day.

Results analysis according to age of patient were based according to older and younger than 40 years according to criteria's by Dandy DJ, Jackson[24,28], those who were younger than 40 years had better results compared to those who are older than 40 years who still complain of mild to moderate pain and intermittent effusion which might be due to associated degenerative changes. Those with pure menisci lesions give better results than those having combined meniscal lesion with plica or tear of ACL, PCL.

Complications were infrequent, thromboembolic disease and septic arthritis are the most dangerous and were not encountered in our study, patient pose to risk of thromboembolic disease, and prophylactic anticoagulant drugs were used.

CONCLUSION AND RECOMMENDATION

A standardized scoring system is needed to properly evaluate and compare arthroscopic menisectomy results and longer duration of follow up is needed to follow patient clinical deterioration and the short duration was behind this limitation.

More studies are required to compare the results of partial and total menisectomy, between medial and lateral meniscal menisectomy and different types of meniscal lesions.

MRI reporting system in our locality dose not provide a valuable diagnostic potential and dose not provide reliable information about the internal condition of the meniscus taking into consideration also the cost effectiveness of the MRI, and improvement of the MRI reporting system is necessary.

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پوختە

ئامانجی چارەسەرکردنی نەخوشیەکانی جومکە وچوك لە بەشی نەشتەگری شکان و ئیسك

تویزینەوەیەکی رابردو – داهاتوو

ئامانج : بەمەبەستی شیکردنەوەی ئەنجامە تایبەتیەکانی تویژینەوەکەمان دوانزە مانگی خایاند ل 1 شواتی 2010 تا 31 کانونی دووەم 2011, دەربارەی پیبینی چۆك بە ئامانجی چارەسەرکردنی نەخوشیەکانی جومگەی چۆك لە بەشی نەشتەرگەری شکان وئیسك لە کولیژی پزیشکی زانکوی هەولیری پزیشکی بۆ ئەو نەخوشانەی کە نشتەرگەری پیبینی چوکیان بۆکرا بۆ ئامانجی دیاریکراو لە نەخوشخانەکانی میری وخومالی هەولیر.

رێگاکان: نەخشەمان کیشابو بۆ تویژینەوەیەکی رابردو_داهاتو بو 160 نەخوش وتەمەنی نەخوشەکان نیوان 21–70 سال وناوندی تەمەنیان 39.5 سال بوو.

ئەنجامەکان : ل کۆی 160 نەخوش , 82 نەشتەرگەری بۆ جومگەی جۆکی راست بوو, 78یش بۆ جومگەی جۆکی چەپ بوو , 113 نەخۆش نیرینە بوون و 47 نەخوشیش میینە بوون, 82 نەخوش برینەکانیان بەهۆی توشبوون لە بواری وەرزشدابووە, 30 نەخوشیش بە هوی کەوتنە خوارەوە لە شوینی بەرزبووە, بەلام رووداوەکانی هاتووچۆ بویتە هۆی توشبوونی 17 نەخوش,کەچی 8 نەخوش توشبوون لە رووداوەکانی کار, لەکاتیکدا 23 نەخوش هوکارەکانی توشبوونی نەزانرابوو.ل کاتی پیبینی دەرکەوت کە پچرانی دواوەی کرکراگەی تەوانەیی.(انسی)دیتراله 61 چۆك وپچرانی لە (قران) پیشەوەی کرکراگەی دیترا لە 30 چۆك, بەلام پچران ل جۆری مسکە قادوس بو کرکراگەی تەوانەی دیترا ل 24 چۆك کەچی خابووەکانی تەوانەی دیترا ل 8 چۆك.

بەلام برینەکانی کرکراگەی تەوانەی وحشی ئەوا پچراوەکانی بەم شیوەی دابەشبوو: لەقەرەنی پیشەوە 14 چۆك بوو,ل قەرەنی دواوە 3 چۆك بوو , مسکە قادوس یەك چوچك بوو, بەلام ئەو برینانەی کە تووشی هەر یەك لە کرکراگەی انسی وحشی گەیشتە 5,6٪, ونشتەرگەری پیبینی بەدی کرد کە 11 نەخوش جومکەی چوچکیان دروست بوو.

دەرئەنجام: نشتەرگەری پیبینی چۆك پیویستە پیش نشتەرگەری برین سازی چۆمکەی چۆك رەچاوبکری بە ئەنجامانی چارەسەرکردنی بە شێوەی ونەخوشیە چەستەییکانی جۆمگە چۆك نەشتەرگەری چۆك پەسەندتر وتایبەتمەندی باشتر ورێژەیەکی کەمی تەشەند لاوەکییەکانی هەیە, هەروەها بنەبرکردنی پشکی کرکراگەی چوچك چارەسەرکردنیکی دایرکراو ورووبە بۆ پچرانی تەنیا لە کرکراگەی تەوانی ناو جۆمگەی چۆك.

هەروەها دۆزینەوەی جۆمکەی چۆك بە پیبینی باشترین هەلبژاردنە بۆ دیاری کردنی نەخوشیەکانی جومگەی چۆك وپیویستە ئەمە راچاو بکری بیش پشکگینینی رەنینیی موگناتیس چونکە پیبینی چۆك ریدەدات بە پیشکەشکردنی هۆیەکانی چارەسەرکردنی لە هەمان کاتدا.

الخلاصة

معالجة الاعتلالات الغضاريف الهلالية بواسطة تنظير الركبة في مستشفيات أربيل.دراسة رجعية مستقبلية

الهدف: تحليل وتقييم نتائج دينا اثني عشر شهرا ممارسة تنظير المفاصل بدءا 1 فبراير 2010 حتى 31 يناير 2011 في قسم جراحة العظام لمريض خضع لعملية تنظير للإدارة الآفات هلالي داخل المستشفيات مدينة اربيل، الحكومية والخاصة.
الطريقة: هذه هي الرجعية – دراسة استطلاعية من 160 المرضى الذين خضعوا لتنظير الركبة للآفات الهلالي في مستشفيات مدينة اربيل. تراوحت أعمار المرضى 21–70 سنة بمتوسط عمر 39.5 سنة.

الهدف من الدراسة: إن الهدف من هذه الدراسة هو تقييم النتيجة على المدى القصير وتحليل البيانات الخاصة بهم وفقا للسن، وتوزيع الجنس، ووضع من الصدمة، والنتائج السريرية، X إشعاعي السينية ونتائج التصوير بالرنين المغناطيسي والتخدير نوع ومدة الإقامة في المستشفى، تنفيذ الإجراءات والنتائج بالمنظار والنتائج السريرية وظيفية قصيرة الأجل الخاصة بهم.
النتائج: تم إجراء تنظير المفاصل على حق 82 و 78 اليسار الركبتين أعراض من 113 من الذكور و 47 من الإناث، تم تصنيف المسببات كما إصابات الرياضة في الركبتين 82 (51.25٪)، وانخفض 30 الركبتين (18.75٪)، حادث مرور 17 الركبتين (10.62٪)، حادث العمل 8 الركبتين (5٪) والامم المتحدة المعروف الركبتين 23 (14.37٪). وكانت النتائج بالمنظار للمرضى المسيل للدموع القرن الخلفي من الغضروف المفصلي الإنسي في 61 الركبتين (38٪)، دلو المسيل للدموع الأمامي القرن الغضروف المفصلي الإنسي في 30 الركبتين (18٪)، الأمامي والخلفي المسيل للدموع القرن الغضروف المفصلي الإنسي في الركبتين 2 (1.25٪)، التعامل مع المسيل للدموع من الغضروف الأنسي في 24 الركبتين (15٪)، المسيل للدموع التنكسية من الغضروف الأنسي في 8 الركبتين (5٪)، والمسيل للدموع القرن الأمامي من الغضروف المفصلي الوحشي 14 الركبتين (8.75٪) والمسيل للدموع القرن الخلفي من الغضروف المفصلي الوحشي 3 الركبتين (1.25 ٪)، دلو مقبض المسيل للدموع من الغضروف المفصلي الوحشي في الركبة 1 (0.6٪) والدموع التي تنطوي على حد سواء هلالات في 6 الركبتين (5.6٪) وهلالات العادية في 11 الركبتين (6.8٪). وارتبطت الآفات هلالي مع تمزق كامل في الرباط الصليبي الأمامي 12 الركبتين (7.5٪)، بتمزق جزئي في الرباط الصليبي الأمامي في الركبة 30 (18٪)، بتمزق جزئي في PCL 3 الركبتين (2٪)، المسيل للدموع كاملة من PCL في 4 الركبتين (2.5 ٪). تأسست الثنية ضيق والمربوطة في 19 الركبتين (11٪) موزعة على النحو الثنية الإنسي الرضفة في 13 الركبتين، الثنية تحت الرضفة – الرباط المخاطية في 5 الركبتين والثنية فوق الرضفة في الركبة 1. وعثر على جثث عظمي غضروفي صغير صغير في الركبتين و2 في 1 المريض كان هناك كيس في الغضروف المفصلي الوحشي.
الخلاصة: بسبب العديد من المزايا ونسبة المضاعفات قليلة وجزئية menisectomy بالمنظار ليكون وسيلة محددة من العلاج لإصابة الغضاريف الهلالية المعزولة من مفصل الركبة. انتعاش سريع ونتائج مبكرة ممتازة بعد الجراحة هي خصائص الأسلوب. أيضا تنظير المفاصل هو الأسلوب المفضل في تشخيص اضطرابات الركبة

PERIODONTAL STATUS IN PATIENTS WITH METABOLIC SYNDROME

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ABSTRACT

Background and objectives: Several studies have reported that metabolic syndrome components such as obesity, hypertension, dyslipidemia and diabetes mellitus are associated with a higher likehood for the occurrences of periodontitis. In the present study, our aim was to evaluate degree of periodontitis in a sample of periodontopathic patients with metabolic syndrome in comparison with those of non-metabolic syndrome and to ascertain the association between degree of periodontitis and metabolic syndrome components.
Methods: This study included three hundred forty patients with periodontitis who attended the Dental Health Polyclinic at a tertiary care teaching hospital in Duhok. After a clinical oral examination, an assessment of the degree of periodontitis and obtaining the demographic data; fasting blood samples were obtained from the patients and glucose, triglycerides and high density lipoprotein-cholesterol levels were estimated. The diagnosis of metabolic syndrome was based on the criteria by the NCEP-ATP III.
Results: Of the 340 patients, 57.9% had mild periodontitis, 39.7% had moderate periodontitis and 2.4% had severe periodontitis. The percent of moderate-severe periodontitis was higher in the metabolic syndrome group compared to the non-metabolic syndrome (45.5% Vs 38.3%0 and showed statistical significance ($p<0.05$).Of the 5 components of metabolic syndrome, patients with moderate-severe periodontitis had higher prevalence of elevated plasma glucose, hypertriglyceridemia and high blood pressure than those with mild periodontitis. patients with 5 components had a higher prevalence of periodontitis (21.4% compared to those without any component of MS (12.1%).
Conclusions: Our study indicates the higher prevalence of moderate-severe periodontitis in patients with metabolic syndrome and thus it necessitates the need for evaluating periodontitis status in patients with metabolic syndrome.

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Key words: Periodontitis, Metabolic syndrome

Metabolic syndrome (MS)is the name for a group of risk factors in one person. The risk factors include abnormal levels of blood lipids(low HDL-cholesterol and high triglycerides), impaired fasting glucose, elevated blood pressure and excess abdominal obesity[1]. Several studies have reported that systemic conditions, such as obesity, hypertension, hyperlipidemia and diabetes are associated with a higher likehood for the occurrences of periodontitis [2,3,4]. An increase in the severity and prevalence of periodontal disease in diabetic patients has been suggested[5]. Pathologic levels of lipidemia may be involved in some of the periodontal status[6]. Epidemiological studies suggested that in some population group, greater pocket depth and a clinical attachment loss are associated with components of the MS[7] . ,and people exhibiting several components of the MS should encourage to undergo a periodontal examination[8]. Regarding obesity and hypertension, the data regarding periodontal status in these conditions are still controversial. The present was carried out to assess the degree of periodontitis in patients with metabolic syndrome in Duhok city population

METHODS

This study was carried out on 340 patients attended the Dental Health Polyclinic, who were diagnosed with periodontitis. The study protocol was approved by the scientific and ethical committees in Duhok City and informed consent was obtained from all the participants at the start of the

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study. The diagnosis of metabolic syndrome was based on the criteria by the American National Cholesterol Education Program Adult III Treatment Panel (NCEP-ATP III) [9] .Patients with MS had at least 3 components of an NCEP-ATP III definition: abdominal obesity (waist circumference>102 cm in men and> 88cm in women), fasting triglycerides level of 150mg/dl or greater, HDL-cholesterol level less than 40mg/dl in men and less than 50mg/dl in women, blood pressure of 130/85 mmHg or greater on pharmacological treatment for hypertension and fasting blood glucose level of 110mg/dl or greater. Patients with a history of kidney disease, liver disease, pregnancy and recent surgery were excluded. At the baseline, the anthropometric data including blood pressure and waist circumference were collected and detailed information on age was done. Assessment of periodontitis was carried out oral teeth excluding the 3' molars. Probing pocket depth (PPD) was measured with a calibrated periodontal probe (Williams probe) at 4 sites (mesiobuccal, distbuccal, mid buccal and mid lingual). Clinical attachment loss(CAL) was assessed by measuring the distance from cemento-enmal junction(CEJ) to the base of the probing pocket depth by using Williams graduated periodontal probe. The patients were classified as mild periodontitis when the CAL 1-2 mm. Patients with CAL 3-4 mm were considered moderate periodontitis and those with CAL>5 mm were severe periodontitis. The participants were asked to fast overnight for at least 12 hours. At the morning, venous blood samples were collected and serum glucose, triglycerides and HDL-cholesterol were determined

RESULTS

This study included 340 patients with periodontitis (178 with MS and 162 non-MS). Their mean age was 42.1+8.8.The general characteristics of patients are presented in (Table 1). Of the 340 patients 57.9% had mild periodontitis and 42.1% had moderate-severe periodontitis. Prevalence of moderate-severe periodontitis was significantly higher in patients with MS and showed statistical significance (45.5% Vs 38.3%, $p<0.05$), compared with those with non-MS, with an odds ratio of 1.34, 95%confidence interval (0.87-2.07).

Table 1 Patient characteristics

Variables	Metabolic Syndrome	Non-Metabolic Syndrome	All
Number	178	162	340
Age(years)	44.4±6.9*	39.5±9.8	42.1+8.8
Male sex[n(%)]	66(37.1)	129(79.6)	195(57.4)
BMI(KG/m 2)	30.9+4.8	26.7+3.8	28.9+4.8
WC(cm)	111.7+10.2	99.3+9.9	105.7+11.8
Hypertension[n(%)]	126(70.8)	57(35.2)	183(53.8)
Diabetes mellitus[n(%)]	114(64.0)	38(23.5)	152(44.7)
Hypertriglyceridemia with lowHDL-ch[n(%)]	125(70.2)	55(34.0)	180(52.9)
Mild Periodontitis[n(%)]	97(54.5)	100(61.7)*	197(57.9)
M-Severe Periodontiti [n(%)]	81(45.5)**	62(38.3)	143(42.1)

** Metabolic syndrome Vs non-metabolic, P<0.01**obtained from x2 test-value <0.05*

(Table 2) shows the association of each component of MS with periodontal status. Of the 5 components of metabolic syndrome, patients with fasting blood glucose of more than 110 mg/dl had a higher incidence of moderate-severe periodontitis as compared to the other components. The odds ratio for this component was (2.53; 95% confidence interval; 1.33-4.82).

Table 2. Association of components of metabolic syndrome with periodontal status

Variables	periodontitis		Odds ratio (95%Cl)
	Mild N (%)	M-severe N (%)	
Waist circumference (cm)			
<90(85)	**65 (58.5)**	**46(41.5)**	
≥90 (85)	**132(57.6)**	**97(42.4)**	**0.64(0.32-1.30)**
Blood glucose (mg/dl)			
<110	**106(56.3)**	**82 (43.6)**	
≥110	**71 (46.7)**	**81 (53.5)**	***2.53(1.33-4.82) Triglycerides (mg/dl)**
<150	**81 (63.8)**	**46(36.2)**	
≥150	**116(54.4)**	**97(45.6)**	**0.84(0.41-1.72)**
HDL-cholesterol (mg/dl)			
≥40(50)	**41 (53.9)**	**35(46.1)**	
<40(50)	**165 (62.5)**	**99(37.5)**	**0.51(0.2-1.33)**
High blood pressure			
No	**96(61.1)**	**61 (38.9)**	
Yes	**61(33.3)**	**122 (66.7)**	**0.96(0.50-1.84)**

(Table 3) shows the distribution of periodontal status by age. Among the different age groups, metabolic syndrome patients in 40->60 year age group had a higher prevalence of moderate-severe periodontitis as compared to the other age groups.

Table 3. Degree of periodontitis in age groups, n (%)

Age (years)	Metabolic syndrome		Non-metabolic syndrome	
	Mild	M-severe	Mild	M-severe
20-30	**5(13.1)**	**0(0.0)**	**24(64.8)**	**9(24.4)**
31-40	**35(30.4)**	**21(18.3)**	**39(33.9)**	**20(17.4)**
41-50	**45(34.3)**	**41(31.3)**	**26(19.8)**	**19(14.4)**
51- >60	**12(21.4)**	**19(33.9)**	**11(19.6)**	**14(25.0)**

(Figure 1) shows the association between MS components and the degree of periodontitis. Patients with 5 components had the highest proportion (16.8%) of moderate-severe periodontitis, compared to those with 4 and 3 components (14.6% and 14.0%) respectively, but the difference was not significant($p>0.05$).(Table 4) shows the distribution of periodontitis in overall patients according to the number of MS components, patients with 5 components had a higher prevalence of periodontitis (21.4% compared to those without any component of MS (12.1%).

Table 4. Distribution of periodontitis in overall patients by metabolic syndrome components

Number of component	Periodontitis N(%)
0	**41 (12.1)**
1	**60 (17.6)**
2	**61 (17.9)**
3	**45 (13.2)**
4	**60 (17.6)**
5	**73 (21.4)**

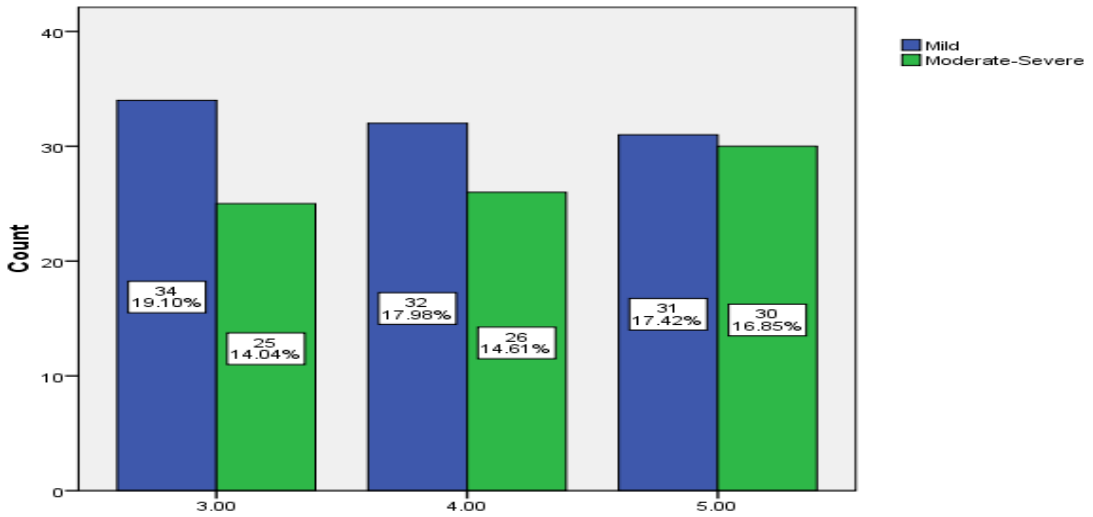

Figure 1. Association of components of metabolic syndrome with degree of periodontitis in metabolic syndrome patients

DISCUSSION

Accumulating evidence suggests that periodontitis may be associated with increased risk of future cardiovascular events and type 2 DM in otherwise healthy individuals [10] . The magnitude of this association, however, seems to be strongly affected by inadequacy of current definitions of periodontitis and the use of multiple clinical criteria to ascertain its grade of severity. It has been reported that systemic conditions, such as obesity, hypertension, hyperlipidemia, and diabetes are associated with a higher likelihood for the occurrence of periodontitis[11].Hence the link between these risk factors and periodontitis create a great incentive to investigate the association between degree of periodontitis and metabolic syndrome components.

To our knowledge, this is the first study to investigate the degree of periodontitis towards metabolic syndrome among periodontopathic patients in a Kurdistan Region population.

The most striking finding of the present study is the high prevalence of moderate-severe periodontitis in patients with metabolic syndrome as compared to the non-metabolic syndrome group. This indicates a possible interplay between the periodontitis status and metabolic syndrome components. The main pathophysiology basis underlying the metabolic syndrome has been attributed to insulin resistance. Insulin resistance is a cardinal feature of type 2 DM and an increased risk of dyslipidemia along with relatively frequently found overweight and obesity.The association between periodontitis and diabetes has been explored by many researchers over the years[12,13] . Periodontal signs and symptoms are now recognized as the 'sixth complication of diabetes" [14] . The general signs and symptoms of periodontitis are the direct result of hyperglycemia and the systemic complications of diabetes mellitus are associated with prolonged hyperglycemia. Thus, blood glucose level plays a key role in the complication associated with diabetes[15].

The present cross-sectional study reports on the periodontal status of periodontopathic patients affected by type2DM. Diabetes mellitus, a common disorder associated with metabolic syndrome, can affect periodontal status, this withstanding 64% of the periodontopathic patients with metabolic syndrome were diabetics as compared to the non-metabolic syndrome group (23.5%).This may reflect the high prevalence of moderate-severe

periodontitis among metabolic syndrome patients. The association between severity of periodontitis and metabolic syndrome also has been suggested by other prospective studies[16,17] For example, in a study by Pozharistkaia et al, [18] it was reported that 66.7percent of the cases with metabolic syndrome had active and aggressive course of chronic generalized showing no components of this syndrome(48.8 %).Analysis of data from 13,170 participants in the NHANES (Third National Health and Nutrition Examination) showed a direct relationship between periodontitis and the prevalence of metabolic syndrome(37% in those with severe periodontitis vs 18% in those with mild or no periodontitis[19]. In the present study, the results were comparable with those of the above mentioned study; the prevalence of severe periodontitis was more in patients with more than three components of the metabolic syndrome. It is noteworthy that 21.4% of the periodontopathic patients appear at risk for increase metabolic syndrome components and a reduction of metabolic syndrome components are associated with low incidence of periodontitis (12.1%). Moreover, the results of this study showed that there were significant differences between periodontopathic patients with metabolic syndrome and non-metabolic syndrome concerning degree of periodontitis.

In 584 Japanese women with 5 components of metabolic syndrome, large waist circumference, low HDL-cholesterol level and high fasting plasma glucose level were associated with significantly higher odds ratios for greater pocket depth values; the adjusted odds ratio for these components were 1.8(95%Cl,1.2-2.8), 2.2(95%Cl,1.4-3.6) and 2.2(95%Cl 1.3-3.9) respectively. The results indicated that metabolic syndrome increase the risk of periodontitis and suggest that people exhibiting several components of metabolic syndrome should be encouraged to undergo a periodontal examination[20] . In comparing the finding of the present study with those of others, the present results exhibit similar outcomes to previous studies. This trend indicates an association between metabolic syndrome components and severity of periodontitis. The present study was not in the agreement with other studies[21] regarding the blood pressure, because the blood pressure was similar between the mild and moderate-severe periodontitis groups. But, however periodontopathic patients appear at increased risk for hypertension and a prevalence of high blood pressure was observed in 183 out of 340 periodontopathic patients (53.8%). Periodontitis can negatively influence certain features of hypertension such as an increase in the left ventricular mass[22]. In general, there has been increasing evidence of a relationship between cardiovascular disease and periodontitis[23], and chronic periodontitis could influence certain features of hypertension such as increased aortic stiffness and increased central blood pressure, which may in turn increase the left ventricular mass in these patients[24]. The various components of metabolic syndrome are all well documented cardiovascular risk factors that co-occur in an individual more often than might be expected by chance. Results of this study indicate that elevated prevalence of hypertension is related to cardiovascular risk factors in periodontopathic patients.. Many studies attempted to examine the relationship between obesity and periodontitis, the majority of these studies were primarily based on analyses of Japanese population and USA data from the third National

Health Examination survey (NHANES). Furthermore, the marjory of the studies used body mass index as indicator of obesity and limited number of studies used combined indicator of overweight and obesity [25, 26]. A strong association was found between body mass index and periodontitis in people with BMI>30Kg/m², it was 3 times more likely

to have periodontitis compared with those of the normal weight[27]. In the present study, BMI was not used as variable by reason of only few patients were obese. Determining waist circumference (WC) eliminates the inconsistencies of BMI. Recent large studies have indicated that measurement of WC or waist-hip ratio may be a better disease risk predictor than BMI[28]. However, there were no significant differences in the percentage degree of periodontitis, percentage of mild or moderate-severe in the highly elevated WC patients as compared to the normal WC group. This result agree with those obtained by Torrungruang et al,[29] , but controversial to the results obtained by Al-Zahrani et al, [30] , they found that WC significantly associated with prevalence of periodontitis(adjusted OR=2.27). In another study, it had been observed that each 1 cm increase in WC was associated 5% increase risk of periodontitis [31] .This difference is because of systematic errors in estimating severity of periodontal disease in the present study and in the other studies. The observed discrepancy in the present data could be to sample derived from general population and not from obese individuals. This discrepancy can also be attributed to variation in the sample size. The investigator contributes that underlying biological mechanisms for the association of obesity with periodontitis are not well-known; however, adipose tissue-derived cytokines and hormones may play a key role. Fat tissue produces a vast amount of cytokines and hormones, collectively called adipokines or adipocytokines which in turn may modulate periodontitis [32]. The association between periodontitis and dyslipidemia has been found in several cross-sectional studies [33, 34]. In the present study, the mean serum triglycerides level was higher in periodontopathic patients with moderate-between dyslipidemia and degree of periodontitis. The study of Baelum,found a 45% prevalence of tooth loss among young age (20-29) year old group to 96% in the severe periodontitis, and although almost all of periodontopathic patients have hypertriglyceridemia, the levels of serum triglycerides were not significantly differed compared with the levels in mild periodontitis group. The Triglycerides level for assessment of dyslipidemia was higher than 150mg/dl in 62.7% of periodontopathic patients, and it was positively associated with prevalence of periodontitis, odd ratio 0.84(95 %Cl 0.41-1.72), although statistically insignificant. The results of this study showed that there was no significant difference between the moderate-severe and mild periodontitis concerning the mean serum HDL-Cholesterol level. However, there were significant differences in the percentage of HDL-cholesterol, percentage of low HDL-cholesterol was higher in the mild periodontitis patients as compared to the moderate-severe periodontitis group. HDL-cholesterol is an important parameter to assess metabolic syndrome status and the majority of the present study population (77.6%) had a reduced HDL-cholesterol. In a study on 261 subjects , it was shown that HDL-cholesterol was directly associated with periodontitis[35] .Such finding was not reported by others, which found no significant statistical result between chronic periodontitis and the plasmatic levels of total cholesterol and cholesterol fractions in study population [36]. This observed difference could be due to the dissimilarity of the study population, the relationship between periodontal condition and lipid metabolism would differ because of the differences in genetic background, diet, population age, sex, structure and body habits [37] .Based on the present data, elevated prevalence of low HDL-cholesterol and hypertriglyceridemia indicate involvement of lipid metabolism in periodontitis. Further research is required to delineate this fascinating link 60 years group. In the present study, the periodontopathic patients in the 20-39 year age had a high prevalence of mild periodontitis as compared to the moderate-

severe periodontitis group [38].The prevalence of moderate-severe periodontitis was higher in patients with age range of 40->60 year. In comparing these findings with those of other studies, the present results exhibit similar outcomes to previous studies. This finding confirms the association between age and periodontitis. The difference in prevalence is explained by the fact that the various metabolic syndrome components such as diabetes mellitus, dyslipidemia and hypertension are more common in older age groups than younger age groups.

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پوختە

رەوشا پدیا لجەم نەخوشێن تەوشا نەساخیا میتابولیزمێ

دەستبیك: زوربەی ڤەکولینا ل سەر پێکهاتێن نەساخیا میتابولیزمێ وەکی قەلەوی , بەرزبونا فیشارا خوینێ , زێدە دوهن , بەرزبونا کولوکوزی یێ گرێدای زێدەبونا هەودانێن گرێن ب پدیێ ڤە .

ئارمانج: زانینا رێژا هەودانێن گرەیێن گرێدای ب پدیێ ڤە لجەم نەخوشێن توشی نەساخیا میتابولیزم یا خوارنێ هەی و بەراوردیەك دگەل نەخوشێن توشی نەساخیا میتابولیزم نەبوین ژبو دلنیاکرن گرێدانا دناڤبەرا هەودانێن گرێیێن پدیێ دگەل میتابولیزمێ .

رێکا کارکرنی: ئەڤ ڤەکولینە ل کومەلگەها تایبەتمەند یا نوشداریا ددانا ل پارێزگەها دهوك . کوردستان (ئیراق) سێ سەدو چل نەخوشا یێن ئاریشەیێن هەودانێن گرێین پدیا ئەڤ فەکولینە لسەر وان بویە . پشتی پشکنینێنا دەڤی , هەلسەنگاندنا پلا هەودانا پدیێ (بەرزەبونا گرێدنا مروڤی , کیوراتیا بەریکێن پدیا) وبدەستڤەئینانا (demographic data) و لسەر خوینا چەند نەخوشێن بروژی ژبو پیڤاناکولوکوزز و دوهنێ سێقولی , کولوسترول پروتینێ دوهنی یێ تیر گەلەك . پشتی هینگێ هاتە دەستیشانکرن کو نەساخێن میتابولیزم لدیڤ ستاندارێن جیهانیە National Cholesterol Education Program – Adult Treatment Panel

ئەنجام: 340 نەخوشا , 57,9 ٪ هەودانێن پدیا هەبوون نە یێن دژوار , 39,7 ٪ هەودانێن پدیا یێن سڤك , 2.4 ٪ هەودانێن پدیا یا دژوار. دەستیشانکرنا دژوار و سڤك ب رێژەکا زور بوون ژ گروپێ نەساخێن میتابولیزمێ (45.5 ٪ 38.3 SV ٪) بەراوردی دگەل ئەوان نەساخێن نە میتابولیزم دیار بو لدیڤ ئامارێن رامانی $p < 0.05$

ژ پێکهاتێن پێنج یێن میتابولیزم ئەوان نەساخێن هەودانێن گرێن دژوار و سڤك دیار بو کو رێژا کلوکوزی یا بەرزە , بەرزبونا دوهنێ سێقولی و بەرزبونا فیشارا خوینێ بەراوردی گەلەك نەساخێن هەودانێن گرێن نە دژوار ئەوان پێج پێکهات هەنە ئەوان رێژەکا بلند ژ گرێن هاریکار بو پدیێ 21.4 ٪ بەراوردی دگەل ئەوێن چ پێکهاتێن میتابولیزمێ نەی 12,1 ٪

دەرئنجام: دیار بو ل بەرهەمیدا کو رێژا بەربەلاڤبونا بلند یا هەودانێن پدیێ لجەم نەساخێن میتابولیزم لەورا یا گرنگە رەوشا پدییا لجەم نەساخێن میتابولیزمێ

الخلاصة

حالة اللثة في المرضى الذين يعانون متلازمة الايض الغذائي

خلفية الموضوع: عدة دراسات اثبتت ان عناصر المتلازمة الايضية مثل البدانة ,ارتفاع ضغط الدم , زيادة الشحوم, مستوى كلوكوز المصل متعلقة بزيادة حدوث التهاب الانسجة الداعمة للثة.

الهدف: معرفة نسبة التهاب الانسجة الداعمة للثة في المرضى الذين عندهم متلازمة الايض الغذائي مقارنة مع المرضى الذين لا توجد عندهم متلازمة الايضية ولتاكيد العلاقة بين نسبة التهاب انسجة اللثة مع المتلازمة الايضية.

طريقة العمل: أجريت هذه الدراسة في مجمع التخصصي لطب الأسنان في مدينة دهوك ،كوردستان (العراق) .ثلاث مائة وأربعون مريضا يعانون من التهاب الأنسجة الداعمة للثة مشمولون في الدراسة . بعد الفحص الفموي ألسريري ، وتقييم درجة التهاب اللثة (فقدان الارتباط البشري ،عمق الجيوب اللثوية)والحصول على demographic data وعلى عينات من دم المرضى الصائمين لقياس الكلوكوز الدهون الثلاثية، كولسترول البروتينات الشحمية العالية الكثافة.وبعدها تم تشخيص مرضى المتلازمة الايضية وفق معايير دولية

National Cholesterol Education Program –Adult Treatment Panel

النتائج: 340 من المرضى، 57.9٪ عندهم التهاب اللثة غير الحاد،39.7٪ عندهم التهاب اللثة المعتدل،2.4٪عندهم التهاب اللثة الحاد– المعتدل .تشخيص الحاد المعتدل كان عاليا في مجموعة المتلازمة الايضية (45.5٪ SV 3 . 38 ٪) مقارنة مع عدم وجود متلازمة الايض وقد اظهر تباين إحصائي معنوي P < 0.0 5 من العناصر الخمسة للمتلازمة الايضية فان المرضى الذين لديهم التهاب الانسجة الحاد المعتدل عندهم نسبة عالية لكلكوز المصل ,ارتفاع الشحوم الثلاثية وارتفاع ضغط الدم مقارنة مع المرضى الذين عندهم التهاب الانسجة غير الحاد.المرضى الذين عندهم خمسة مكونات عندهم نسبة عالية لالتهاب الانسجة الداعمة للثة 21.4٪ مقارنة مع الذين لا توجد عندهم اي مكونات للمتلازمة الايضية 12.1٪

الاستنتاج: أظهرت النتائج أن هناك نسبة انتشار عالية من التهاب اللثة في مرضى المتلازمة الايضية ولهذا فمن الضروري تقييم حالة اللثة في مرضى المتلازمة الايضية .

EFFECTIVENESS OF A HEALTH EDUCATIONAL PROGRAM ON KNOWLEDGE OF PATIENT WITH MYOCARDIAL INFARCTION

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ABSTRACT

Background and objectives: Myocardial infarction is the death of myocardial tissue as a result of prolonged lack of blood and oxygen supply. Knowledge about causes, risk factors and lifestyle, has impact on the life of patients. The objective is to assess knowledge of myocardial patient before and after implementing of health educational program about heart attack (pre and post-test).

Methods: A quasi-experimental study was carried out through the application of pre-test and post-test, on patient with myocardial infarction at Hawler and Rizgary teaching hospitals in Erbil city. The study sample consist of (303) patients. The data collections were carried out from the period of the 1st of May 2008 to the 1st of May 2009.

Results: The highest percentage (30.36%) of the study samples their age between (60-69) years, more than half of the study samples were illiterate. The majority (86.13%) of the study samples were not received any information about MI. There were highly significant differences of patient's knowledge in the pre and post-test. As well as in relation between patient's knowledge and their age, level of education and occupation between the pre and post-test.

Conclusion: The findings revealed that the health educational program has an effect on increasing the knowledge of the patients with myocardial infarction in the post-test.

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Key words: Health educational program, knowledge, myocardial infarction.

Cardiovascular disease it remains the major cause of mortality in the twenty-first century, nearly one have of all deaths in industrialized nations and 25% of those in developing countries are due to coronary heart disease[1].

In the United Kingdom annually, it kills more people than the next five causes of death combined including cancer, chronic lower respiratory disease, accidents, diabetes, influenza and pneumonia[2]. Various lifestyle factors such as, cigarette smoking, lack of exercise, and unhealthy diet , abdominal obesity, high serum lipid level, high blood pressure and diabetes mellitus are risk factors of CVD [3]. A study conducted in Iraq that the prevalence of cardiac disease is12 per 1000 persons, rates are slightly higher in Urban area than in rural areas 9.2 per 1000 persons and it increased after the age 50 years [4]. To prevent the onset and recurrence of myocardial infarction, the most important thing is to control the risk factors of atherosclerosis, the main cause of myocardial infarction[5]. A study was found that a high prevalence of cardiovascular disease, risk factors among a sample of 5840 Omanian their age was 20 and older [6].

In Erbil a survey showed that urban population has the same cardiovascular disease risk factors profile as western societies including hypertension, diabetes mellitus, and smoking[7]. Many preventive measures can be taken to avoid heart attack. Controllable factors that can contribute to the heart disease which include smoke, high blood pressure, high serum cholesterol, stress, obesity, sedentary lifestyle and diabetes mellitus[8]. Improvements in diagnosis, prevention and treatment, the mortality rate from coronary heart disease has declined gradually over the past several decades[9].

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METHODS

A purposive sample consist of 303 patients who have myocardial infarction admitted to the CCU in Hawler & Rizgary Teaching hospitals, have taken as pre and post-test sample. In the post-test 17 of them were not included because 7 patients were dropout from the study in the post-test, and 10 of patients were died. Thereby 286 patients remain in the post-test from both setting. Data were collected through interview questionnaire method from the period of the1st of May 2008 to the1st of May 2009. Before starting educational program, the researcher introduced himself to the patient then explains the process of research work. when the patient accepted to be included in the study, the researcher start to collect the data through questionnaire which including socio-demographic and medical data, patient's knowledge about their condition as pre-test (before implementing the health educational program), then the researcher expose the patients to an individualized a health educational program when patient's condition has been stabilized. Information was given verbally in a structured way using a pamphlet developed by the researcher. It contains; nature of disease, knowledge about causes, risk factors and clinical manifestations. The criteria of the study were patient with myocardial infarction, admitted in the CCU in the hospitals, both gender and accept to participation in the study. Pilot study was carried out in both hospitals which consist of 20 patients and the reliability were 0.97. Data were analyzed through the application of the (Statistical package for social science SPSS, version 17, t. test).

RESULTS:

(Table 1) shows the highest percentage (30.36%) of the study sample's age were (60-69) years. The majority of them were male. and illiterate. Moreover, the highest percentage (35.97%) of them were house worker.

Table 1. Socio-demographic characteristics on 303 patients with acute myocardial infarction.

No	Variables	Total n=303	
1	Age	F	%
	20 -29 years	3	0.99
	30- 39 years	14	4.62
	40- 49 years	34	11.22
	50- 59 years	89	29.37
	60- 69 years	92	30.36
	70- 79 years	62	20.46
	80 and above	9	2.97
2	Gender		
	Male	196	64.68
	Female	107	35.32
3	Level of education		
	Illiterate	201	66.33
	Read and write	37	12.21
	Primary school graduate	49	16.17
	Secondary school graduate	9	2.97
	Institutional graduate	4	1.33
	College graduate and more	3	0.99
4	Occupation		
	House worker	109	35.97
	Governmental employee	31	10.24
	Free occupation	84	27.72
	Retirement	79	25.74

While (Table 2) shows more than half of the study sample interviewed in the second day of admission and most of them admitted to the hospital in the first 12 hours of heart attack. 48.85% of them expose chest pain in the morning with severe chest pain in the central, with inferior myocardial infarction and (57.75%) of them were having heart attack for the first time.According to the patient's risk factors, the majority (87.1%) of the study sample were aging, it mean that aging is the major risk factors of myocardial infarction. Table (4) shows that (32.5%) of the study sample they knew that myocardial infarction is necrosis of myocardial tissue in the post-test, while in the pre-test were (3.6%).

Table 2. Medical Data on 303 patients with acute myocardial infarction.

No	Medical data	Total n= 303	
1.	Day of interviewing	F	%
	First day of admission	7	2.32
	Second (lay of admission	177	58.41
	Third day of admission	119	39.27
2.	Time of hospitalization		
	First 12 hours of chest pain	186	61.39
	More than 12 hours of chest pain	117	38.61
3.	Time of chest pain		
	Morning	148	48.85
	Evening	59	19.47
	Night	96	31.68
4.	Intensity of chest pain		
	Mild	17	5.61
	Moderate	56	18.49
	Severe	230	75.9
5.	Location of pain		
	Central	186	61.39
	Substernal	56	18.48
	Epigastric	40	13.2
	Back pain	21	6.93
6.	Zone of MI		
	Inferior MI	154	50.83
	Anterior MI	124	40.92
	Extensive MI	13	4.29
	Anterolateral MI	9	2.97
	Posterior MI	3	0.99
7.	Number of heart attack		
	First	175	57.75
	Second	78	25.74
	Third and more	50	16.51

Also (40.5%) of them knew the cause of myocardial infarction is arteriosclerosis in the post-test, while (8.6%) in the pre-test. Regarding risk factors (99%) of the patient were knew that salty diet intake is the risk of myocardial infarction in the post-test, while (80%) in the pre-test. About clinical manifestation during heart attack (96.5%) of the sample were knew that nausea and vomiting are clinical manifestation in the post-test, compared to (80%) in the pre-test. Whereas (12%) of them knew that drinking of cold water aggravated chest pain in the post-test, while (6.6%) in the pre-test.

The statistical comparison in the table (5) shows highly significant between pre and post-test of patient's knowledge regarding

the disease which include; meaning of myocardial infarction, causes, risk factors, clinical manifestation during heart attack and factors aggravating chest pain $p< 0.05$

Table 3. Risk factors on 303 patients with acute myocardial infarction.

NO.	Risk factors according patient 's chart	NO.	%
1.	Diabetes mellitus	116	38.3
2.	Obesity	34	11.2
3.	Hypertension	194	64.1
4.	Previous stroke	17	5.6
5.	Smoking (tobacco)	179	59.1
6.	Hyper cholesterol	135	44.5
7.	Ischemic heart disease	104	34.3
8.	Family history-first degree	165	54.4
9.	Family history-second degree	50	16.5
10.	Aging	264	87.1

Table 4. Distribution and percentage of myocardial infarction patient's knowledge in the pre and post test

#	Items	Pre-test n=303				Post-test n=286			
		Know		Do not Know		Know		Do not Know	
1.	Meaning of myocardial infarction	no	%	no	%	no	%	no	%
A	Occlusion of coronary arteries	91	30	212	70	199	69.5	87	30.5
B	Necrosis of myocardial tissue	11	3.6	292	96.4	93	32.5	192	67.5
2.	Causes of MI								
A	Atherosclerosis	62	20.5	241	79.5	122	42.6	164	57.4
B	Arteriosclerosis	26	8.6	277	91.4	116	40.5	170	59.5
3.	Risk factors of MI								
A	Hypertension	124	40.9	179	59.1	244	85	42	5
B	Diabetes mellitus	104	34	199	66	239	83.5	47	16.5
C	Smoking tobacco	183	60.4	120	39.6	267	93.3	19	6.7
D	Hyper cholesterol	38	12.5	265	87.5	88	30.7	189	69.3
E	Abdominal obesity	17	5.6	286	94.4	116	40.5	170	59.5
F	Excessive alcohol consumption	6	2	297	98	41	14	245	86
G	Some drugs (codeine & NSAID)	18	6	285	94	62	21.6	224	78.4
H	High saturated fatty diet intake	223	73.6	80	26.4	277	97	9	3
I	Salty diet intake	243	80	60	20	284	99	2	1
J	Stress	220	72.5	83	27.5	280	98	6	2
K	Family history	45	15	258	85	159	55.6	127	44.4
L	Aging	24	7.9	279	92.1	140	49	146	51
M	Gender	7	2.3	296	97.7	20	7	266	93
4.	Clinical manifestation during heart attack								
A	Chest pain	289	95	14	5	285	99.6	1	0.4
B	Dyspnea	242	79.8	61	20.2	280	98	6	2
C	Nausea & vomiting	243	80	60	20	276	96.5	10	3.5
D	Excessive sweating	256	84.4	47	15.6	277	96.8	9	3.2
E	Palpitation	88	29	215	71	143	50	143	50
F	Syncope	42	24	261	86	88	30.7	198	69.3
5.	Factors aggravated chest pain								
A	Heavy work	192	63.4	111	36.6	257	90	29	10
B	Stress & emotion	232	76.5	71	23.5	278	97	8	3
C	Heavy meals	69	22.8	134	77.2	139	48.6	147	51.4
D	Drink cold water	20	6.6	183	93.4	35	12	251	88

Table 5. Statistical comparison between the pre and post test of MI patient's knowledge

#	Knowledge	Pre-test n=303		Post-test n=286		p-value of t-test
1.	Meaning of myocardial infarction	M	SD	M	SD	
A	Occlusion of coronary arteries	1.3	0.45	1.7	0.46	0.000
B	Necrosis of myocardial tissue	1	0.19	1.3	0.47	0.000
2.	Causes of MI					
A	Atherosclerosis	1.2	0.41	1.4	0.5	0.000
B	Arteriosclerosis	1	0.28	1.4	0.5	0.000
3.	Risk factors of MI					
A	Hypertension	1.4	0.5	1.8	0.36	0.000
B	Diabetes mellitus	1.3	0.5	1.81	0.37	0.000
C	Smoking tobacco	1.6	0.5	2	0.25	0.000
D	Hyper cholesterol	1.13	0.33	1.3	0.46	0.000
E	Abdominal obesity	1	0.23	1.4	0.5	0.000
F	Excessive alcohol consumption	1	0.14	1.14	0.35	0.000
G	Some drugs (codeine & NSAID)	1	0.24	1.2	0.41	0.000
H	High saturated fatty diet intake	1.7	0.44	2	0.2	0.000
I	Salty diet intake	1.8	0.41	2	0.08	0.000
J	Stress	1.7	0.45	2	0.14	0.000
K	Family history	1.15	0.36	1.6	0.5	0.000
L	Aging	1.1	0.3	1.5	0.5	0.000
M	Gender	1	0.15	1.1	0.26	0.000
4.	Clinical manifestation during heart attack					
A	Chest pain	1.9	0.21	2	0.06	0.000
B	Dyspnea	1.8	0.4	2	0.14	0.000
C	Nausea & vomiting	1.8	0.4	2	0.18	0.000
D	Excessive sweating	1.8	0.33	2	0.18	0.000
E	Palpitation	1.3	0.5	1.5	0.5	0.000
F	Syncope	1.1	035.	1.3	0.5	0.000
5.	Factors aggravated chest pain					
A	Heavy work	1.6	0.5	2	0.3	0.000
B	Stress & emotion	1.7	0.42	2	0.17	0.000
C	Heavy meals	1.2	0.42	1.5	0.5	0.000
D	Drink cold water	1	0.25	1.2	0.33	0.000

DISCUSSION

This study tested myocardial infarction patients' knowledge. Age is one of the risk factors that cannot be altered with increasing the age 10. This study found that (30.36%) of the study samples were within age group (60-69) years, most of them were illiterate. A study found that coronary artery disease is more common among people with less education [11].

The study shows that (35.97%) of the study samples were house worker. A study conducted on 187 myocardial infarction patient that (47.1%) of them worked under difficult condition [12].

Whereas more than half of the study sample were interviewed in the 2nd day of admission and most of them admitted to the hospital in the first 12 hours of heart attack with severe central chest pain in the morning, about half of them they have inferior MI and (57.75%) of them were having first heart attack. In contrast a study found, that those who have had anterior MI have twice risk for death of patient than who have had inferior MI [13].(Table 4) shows that (69.5%) of the study sample knew the meaning of occlusion in the coronary artery disease (CAD) is myocardial infarction, compared to (30%) in the pre-test. Also (42.6%) of them they knew the cause of MI is atherosclerosis, compared to (20.5%) in the pre-test and 98% of them they knew that stress is risk of MI in the post-test compared to (72.5%) in the pre-test. A prospective study found

that negative stress and emotion are risk factors for the incidence of coronary artery disease [14,15]. Nearly the entire study sample knew that dyspnea is a clinical manifestation in the post-test compared to (79.8%) knew in the pre-test. While nearly half of them they knew that heavy meals is aggravated chest pain in the post-test compared to (22.8%) in the pre-test. A study found that up to 30% of MI patients are known to have CAD and such patients at risk of coronary events and their close relatives, would benefit from appropriate advice perhaps in the form of written guidelines [16]. A study has shown that low levels of educations are associated with premature MI [17, 18.]

There were highly statistical differences between pre and post-test of patient regarding myocardial infarction disease.

CONCLUSION

Myocardial infarction patients improved by giving health educational program which lead to increased knowledge of all patients regarding myocardial infarction in the post-test.

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پوخته

هەڵسەنگاندنی زانیاری نەخۆشی تووش بوو بە نۆرەی دڵ لە سەر پرۆگرامی ڕۆشەنبیری تەندروستی

زانیاریو ئامانجەکان: نۆرەی دڵ بریتیە لە مردنی شانەکانی ماسولکەی دڵ بە هۆی نە گەیشتنی خوێن و ئۆکسجین بۆ ماوەیەکی زۆر. زانیاری دەربارەی هۆیەکان و هۆکارە ترسناکەکان و شێوازی ژیان , کاریگەری هەیە لە سەر ژیانی نەخۆش. ئامانجەکان بریتیە لە هەڵسەنگاندنی زانیاری نەخۆشی تووش بوو بە نۆرەی دڵ لە سەر پرۆگرامی ڕۆشەنبیری تەندروستی (پێش و پاش تێست).

ڕێگا: توێژینەوەیەکی نیمچە ئەزموونیە جێ بە جێ کرا بە شێوەی ئەنجامدانی تێستی پێش و پاش لەسەر نەخۆشی تووش بوو بە نۆرەی دڵ لە نەخۆشخانەکانی هەولێر و ڕزگاری فێرکاری, ژمارەی نمونەکان (303) نەخۆش بوون. زانیاریەکان کۆکرانەوە لە ماوەی نێوان 1-5-2008 تاکو 1-5-2009.

ئەنجامەکان: بەرزترین ڕێژەی سەدی (30,36٪)لە نمو نەکان تەمەنیان لە نێوان (60-69) سالّی بوون, زیاتر لە نیوەی نمونەکان نەخوێندەوار بوون, زۆربەی نمونەکان (80,13٪) زانیاریان نەبوو لەسەر نۆرەی دڵ. جیاوازیەکی گرینگی بەرز هەبوو لەسەر زانیاری نەخۆش لە ئەنجامدانی تێستی پێش و پاش, هەروەها پەیوەندی هەیە لە نێوان زانیاری نەخۆش لەگەڵ تەمەنیان و ئاستەکانی خوێندەواری و کارەکانیان لە ئەنجامدانی تێستی پێش و پاش .

کۆتایی: دۆزینەوەکە دەگەڕێتەوە بۆ کاریگەری بەرنامەی ڕۆشەنبیری تەندروستی لەسەر زیاد کردنی زانیاریەکان بۆ نەخۆشی تووش بوو بە نۆرەی دڵ لە ئەنجامدانی تێستی پاشینە.

الخلاصة

تقييم المعلومات للمرضى المصابين باحتشاء القلبية قبل و بعد تطبيق برنامج التثقيف الصحي

الخلفية والاهداف: احتشاء العضلة القلبية هي موت لانسجة عضلة القلب نتيجة لعدم وصول الدم و الاوكسجين لفترة طويلة. المعلومات حول الاسباب و العوامل الخطرة و نمط الحياة له تاثير على حياة المريض. الاهداف هو تقييم المعلومات للمرضى المصابين باحتشاء القلبية قبل و بعد تطبيق برنامج التثقيف الصحي (اختبار القبلي و بعدي).

منهجية البحث:هي دراسة شبه تجريبية نفذت من خلال تطبيق الاختبار القبلي والبعدي على المرضى المصابين باحتشاء العضلة القلبية في مستشفيات التعليمية اربيل و رزكاري. عدد العينات تحتوي على (303)مريضاً، جمعت المعلومات خلال الفترة من 1-5-2008 الى 1-5-2009.

النتائج: أعلى نسبة المئوية (30,36٪)من العينة كانت عمرهم بين (60-69) سنة، اكثر من نصف العينة كانوا أميين, غالبية العينة (80,13٪) ليس لهم معلومات عن احتشاء العضلة القلبية. هناك فروقات معنوية عالية لمعلومات المرضى في اختبار القبلي و البعدي و كذلك هناك العلاقة بين معلومات المريض مع عمرهم و مستوى تعليمهم و عملهم قبل و بعد الاختبار.

الأستنتاجات: بينت النتائج بأن برنامج التثقيف الصحي له تأثير على زيادة معلومات للمرضى المصابين باحتشاء العضلة القلبية في الاختبار البعدي.

EFFECT OF ALENDRONATE ON SERUM GHRELIN LEVEL IN OSTEOPOROTIC POST MENOPAUSAL WOMEN

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ABSTRACT

Background: Osteoporosis is a systemic skeletal disorder, characterized by low bone mass and micro-architectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk. In humans, changes in estrogen status due to advancing age and menopause have been associated with bone loss. Ghrelin is a novel peptide hormone consist of a (28) -amino acid primarily synthesized by the stomach and released in response to fasting, and this hormone play role in bone physiology.
Objective: This study was designed to evaluate the effect of the antiresoptive a bisphosphonate(BPs) drug "Alendronate tablet" on serum ghrelin level.
Methods: Twenty three postmenopausal women with(mean ± SD age, 64.3±8.3y), diagnosed as osteoporotic patients by measuring the bone mineral density (BMD) by Dual x-ray absorptiometry (DXA) and treated by Alendronate tablets (70mg/once weekly) for three months during1st November 2011 to 1st March 2012 .The study was conducted in Ibn Sina Teaching hospital in Mosul city. Serum ghrelin hormone concentration was measured before and after treatment with a commercial Enzyme-Linked Immune Sorbent Assay (ELISA) kit.
Results: This study showed that three months treatment with alendronat drug led to a statistical significant ($p<0.05$) increment (21.41%) in the basal serum level of ghrelin hormone and there was a significant inverse correlation between ghrelin and body mass index(BMI).
Conclusion: This study concluded that alendronate treatment have lead to a statistical significant increase in the serum ghrelin level in osteoporotic post-menopausal women.

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Key words: Osteoporosis, Post-menopause women, Alendronate, Ghrelin hormone

Bone is a living tissue and throughout life, the skeleton is continually remodeled in an orderly sequence of bone resorption followed by bone formation [1]. The two major determinants of risk in the development of osteoporosis are peak bone mass and the rate of bone loss [2]. During periods of growth, the rate of bone formation exceeds that of resorption and the reverse is true with ageing [3].

Osteoporosis results from a disturbance in the remodeling process in which the net rate of bone resorption exceeds the rate of bone formation which brings about characteristic decrease in bone mass [4]. It can occur not only from loss but also from failure earlier in life to make sufficient bone resulting in lower peak bone density at skeletal maturity [2]. Peak bone density is the maximum bone mass reached in life between age20-35years [4]. Sex hormones plays a role in bone turnover in addition to mechanical and gravitational forces, for example, bone loss occurs with ageing as a consequence of oestrogen deficiency in postmenopausal women, as well as through oestrogen-independent, age-related mechanisms, that may involve secondary hyperparathyroidism and reduced mechanical loading[1].

According to the WHO study group (1994)5, osteoporosis in post menopausal women was defined in terms of BMD measurement and based on comparison of the patients measurement to the standard peak adult bone mass as follow;"osteoporosis is present when the BMD is more than 2.5 standard deviations (SD) below that of healthy pre menopausal adult females "T - score"6. BMD values can be estimated for various skeletal sites,

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and the values are expressed in grams of hydroxyapitite per sequre cm2 (g/cm2) of the area scanned by DXA[2].

The management of osteoporosis is intended to prevent fractures and maximize skeletal strength[3]. A large number of drugs have been used to treat osteoporosis, and the patterns of use vary greatly from country to country, the choice of agent will depend not only on effectiveness of the treatment but also on other considerations such as side effects, cost and availability [2].

Bisphosphonates are the most commonly prescribed medications for the treatment of osteoporosis [7].

Alendronate is a nitrogen-containing bisphosphonate, which is prescribed since September1995, has the greatest amount of efficacy and safety [8]. It reduces pain and improves quality of life more effectively [9.] It is available in oral form(tablet) 10mg/day, 35mg twice weekly and 70mg/once weekly [7]. It is a potent inhibitor of bone resorption in vitro, recently, it has been reported that alendronate stimulates osteoblast differentiation while inhibiting adipogenesis in vitro [10].

Ghrelin is a peptide hormone consist of 28 amino acids found in mammalian species as well as non mammalian species. It was identified as an endogenous ligand for an orphan receptor termed growth hormone secretagogue receptor GHS-R, which induces release of growth hormone (GH) from the pituitary via the hypothalamus, as well as by direct action on the pituitary [11]. Ghrelin had a multifunctional effects including regulation of feeding behavior [12], increasing GI motility[13], control of cell proliferation [14], hormone secretion [15]and modulation of the reproductive axis[16]. Recently, ghrelin peptide was reported to play an important biological role in metabolism of bone [17].

Ghrelin is produced by human bone cells and it was found to promote proliferation of human osteoblast via a mechanism that involves activity of the mitogen-activated protein (MAPK) and phosphoinositde 3-kinase PI3K signaling pathways, although these cells express only the GHS-R1b isoform, which is considered to be inactive[18].

The role of ghrelin in bone physiologically reinforced by additional observation; includes the systemic ghrelin secretion correlation with BMD in healthy adolescent [19]. Decreased systemic ghrelin might be associated with osteopenia after gastrectomy and this may be attenuated by either ghrelin treatment or retaining part of oxyntic gland area [20]. These observations indicate that ghrelin may stimulate bone formation directly and that it may be useful in the treatment of either osteoporosis or metabolic bone disease content [21].

METHODS

This study was conducted at the rheumatology and rehabilitation department in Ibn Sina Teaching Hospitals in Mosul city. Data collection was covered a period of four months from 1ST November 2011 to 1ST March 2012. This study was carried out on twenty three postmenopausal women , diagnosed as osteoporotic with BMD measurements by using DXA (Hologic, model ASY-00409, 2008USA), with a T-score -2.5 or less, their ages was ranged between 63 to 70years (mean+SD 64.3+8.3 years). The patients in this study were provided with Alendronate tablet (PMS-Alendronate®, Canada). The dose was 70mg once weekly, and all patients were followed for three months. Patients included in this study were osteoporotic postmenopausal women had diabetes mellitus , hyperlipidemia, and hypertension.

Two blood samples were collected; first sample at baseline before starting treatment with Alendronate tablet, and the second sample was collected three months after treatment. Five milliliters of blood was drawn by venipuncture and collected in plain tube kept at room temp for 30 minutes and centrifuged for 15 minutes

(Remi moter, china) at 1000 rpm. Then serum samples were removedand transferred to new two eppendroff tubes to be stored at -20 °C or -80°C.

Serum ghrelin hormone was measured with a commercial Enzyme-Linked Immune Sorbent Assay (ELISA) kit according to the manufacturer's (My Biosour,U.S.A) and the report of Germain et al (2009) 22. The height (in m) was measured with wall stadiometer; weight (in kg) was measured with an electronic scale (in light street clothing and without shoes). (BMI) was calculated by Quetelet equation (dividing weight in kilogram (Kg) by the square of height in meters $(m)^2$.

All values quoted as the mean± standard deviation (SD), the data were analyzed using One-sample and paired-sampled Student's t-test to compare the difference between before and after treatment samples for ghrelin concentration. Regression analyses among variables were tested using Pearson correlation coefficient. Analysis were done using SPSS version 11. Differences between observation were considered significant at $p<0.05$.

RESULTS

(Table 1) The baseline characteristics of postmenopausal women in the study. In (Table 2) the mean serum ghrelin hormone concentrations in post treatment osteoporotic patient with Alendronate tablet (23.93±19.44ng/ml) was significantly higher than those of pre treatment level (19.17± 18.73ng/ml) ($p>0.01$).

Table 1. Demographic characteristics of postmenopausal women

Parameters	Study group	P
N	23	
Age (year)	64.3±8.3	0.01**
Weight(kg)	71±11.7	0.01**
Height(m)	150±6.1	0.01**
BMI kg/m2	31.16±5.06	0.01**
BMD(g/cm2)	0.79 ± 0.08	0.01**
T-score	-2.58 ± 0.6	0.01**

**Data represented as mean ± SD.*
*** Significantly different (p<0. 01)*

Table 2. Serum ghrelin levels in before and after Alendronate treatment

Parameters	Follow up (pre)	Follow up (post)	P =
N	23	23	
Ghrelin conc.(ng/ml)	19.17± 18.73	23.93±19.44	0.01**

*** Significantly different (p<0. 01)*

(Table 3) represents the outcomes of Pearson's correlation coefficient A. significant inverse correlation was found between the BMI of the patient with pre treatment serum ghrelin concentration ($r = -0.418$, $p < 0.05$). No significant correlation was found between the BMD, and height with pre and post serum ghrelin concentration. Weight and age were negatively correlated with pre and post serum ghrelin concentration but not statistically significant.

Table 3. Correlation between different parameter and serum ghrelin level before and after treated women

Parameters	Ghrelin before	p	Ghrelin after	P
Age (year)	-0.202	0.355	-0.054	0.837
Weight(kg)	-0.362	0.09	-0.353	0.099
Height(m)	0.076	0.739	0.074	0.737
BMI kg/m2	-0.418*	0.047	-0.410	0.052
BMD(g/cm2)	0.293	0.174	0.315	0.144
T-score	0.237	0.277	0.225	0.301

*** Correlation is significant at the 0.01 level (2-tailed).*
** Correlation is significant at the 0.05 level (2-tailed).*

DISCUSSION

Laboratory studies suggested that ghrelin is involved in bone metabolism, but studies of ghrelin and bone in women are limited[14].

Alendronate is a potent bone resorption inhibitors are thought to increase bone mass by filling in the 'remodeling' space [7]. Osteoclastic resorption lasts about three weeks after treatment and is followed by osteoblastic bone formation which lasts three to four months [4], therefore we evaluated three months for the study to show the effect of Alendronate on ghrelin level in the stage of osteoblast bone formation in postmenopausal women. In vitro studies showed that, alendronate directly stimulate the differentiation and bone forming activity of osteoblasts[23, 24]. Recently, it has been reported that alendronate stimulates osteoblast differentiation while inhibiting adipogenesis in vitro [10].

In the present study serum ghrelin hormone concentrations in post treatment osteoporotic patients was significantly higher than those of basal level before treatment with Alendronate. In vitro studies showed the effects of ghrelin in primary osteoblast-like cells and cell lines, with highly variable responses in proliferation, differentiation, and survival assays[18]. Furthermore ghrelin increased alkaline phosphatase (ALP) activity and mineralization in osteoblast cell in addition to elevating the expression of several osteoblast differentiation markers like Collagen[1]ALP and osteocalcin (OC) "marker of bone formation" and Ca+2 accumulation in matrix [25].

Fukushima and colleagues showed that ghrelin also promotes bone formation in vivo by administering peripheral ghrelin to rodents over a 4 weeks period and demonstrating increased BMD[26]. They also showed that peripheral ghrelin given to genetically GH-deficient spontaneous dwarf rats (these rodents lack GH and thus the GH-IGF-1 axis) increases BMD, suggesting that ghrelin can act on bone independent of GH.

Osteoblast differentiate from mesenchymal cell (MSCs) of bone marrow, the differentiation of MSCs towards the osteoblast lineage is mediated by different cytokines, hormones and growth factors; fibroblast growth factor, transforming growth factor, bone morphogenetic protein , insulin-like growth factor (IGF), parathyroid hormone and glucocorticoids, vitamin D and estrogen[27].

Ghrelin as a growth hormone secretagogue both in vitro and in vivo is likely to have a direct effect on growth hormone increases [28]. Under normal conditions endogenous ghrelin indirectly increases stimulates IGF-1 release from the liver by stimulating its own receptor [29]. GH is considered essential for both the growth and maintenance of skeletal mass. In vitro, GH stimulates osteoblastic proliferation, differentiation, and matrix mineralization [30]. Elderly postmenopausal women have lower GH secretory amplitudes and reduced serum

levels of IGF-I and IGFBP compared to younger adults and likely to cause age-related metabolic and physiological dysfunction, moreover, the pulse frequency for GH is less in older people [31].

Unfortunately, we did not measure serum GH level, but a 24 –hour profile of human plasma GH concentration resembles that of ghrelin [32]. The clinical studies showed a relationship between GH and circulating ghrelin [33].

Study have shown significant inverse correlation was found between the BMI of the patients and serum ghrelin concentration , lower ghrelin concentrations in obese subjects and who have high BMI, these results were in agreement with[32,34,35]. Fagerberg and colleagues reported that ghrelin level negatively correlated with body fat and waist circumference[36]. Body fatness was the strongest determinant of circulating ghrelin level. BMI does not indicate body fat directly, but research has shown that BMI is correlated with direct measures of body fat[37].

Furthermore, there is no correlation was found between the BMD and serum ghrelin level. Researchs studying the association between ghrelin and BMD is limited, but only one study in rats found that ghrelin was positively correlation with BMD[26], but another study in mice found no correlation . This study is in agreement with the human study in adult which found that ghrelin were not correlated with BMD at femoral neck or lumbar spine in 80 Korean men [39]. Other study showed that systemic ghrelin secretion correlation with BMD in healthy adolescent[19]. In the other study a significant negative correlation between ghrelin and BMD was observed in younger women[34]. In fact, in animal studies gastrectomy has been reported to induce osteopenia by reducing ghrelin secretion; such osteopenia can be reversed by ghrelin treatment[40]. In humans, after laparoscopic gastric bypass surgery for morbid obesity, a decrease in serum ghrelin levels and an increase in bone resorption, with a subsequent reduction in bone mass, have been detected[41].

In our study we did find inverse correlation between ghrelin and age but statistically not significant. Studies to determine whether ghrelin levels change during aging have been inconclusive. For example, Rigamonti et al reported a decline in fasting ghrelin levels in elderly humans compared with young controls[42], but Sturm et al found that, ghrelin levels were the same in young and elderly females[43]. Recent studies showed that mean -h acyl-ghrelin levels are lower in a group of adults' age 60 years and older when compared to a group of young adult's age 30 years and younger where both groups had a similar BMI[44]. The pituitary ghrelin receptor content does not decline with aging [45], and the secretary response of the pituitary to ghrelin and GH secretagogue is sustained in the elderly [46].

Conclusion: Alendronate treatment leads to increase in serum ghrelin level. No comparable study is available in this regard and further studies are needed to investigate the mechanism responsible for this finding.

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پوخته

تاسیری دەرمانی الدرونێت ل سەر ڕێژەی هرمونی گریلین ل ئافرەتانی توشی نەرمبوونی ئیسك

پێشەکی:نەرم بوونی ئیسك جورە نەخوشیکە تووسی کوئەندامی ئیسکە پیکەر دەبیت,لەم ئیشە بارستەی ئیسك کەم دەبیتە وە و مەترسی شکاندنی ئیسك زیاتر دەبیت. ل مروۆ دا ئەو گۆرنکاری ل هرمونی ئیستروجین رووەدات لگەل زیادبوونی تەمەنی مروۆ و بە تایبەتی ل ئافرەت دا دەبیتە هوکاری کیم بوونی بارستەی ئیسك و زیاد یوونی مەترسی شکاندنی ئیسك ب ئەگەری رووداوەکی بچووك. هرمونی گریلین جورە هرمونی نوی یە پێکدیت ل (28) ترشی أمین و ل گدە دروست دەبیت لە کاتی بە رۆژیبون(نان نەخواردن). هرمونی گریلین رۆلەکی بەرزی هەیە ل دروست بوونی ئیسك و بنایت دانەوەی ئیسك.

ئامانج: ئەم توژینەوە دیراسەت کردنی تاسیری دەرمانی الدرونێت ل سەر ڕێژەی هرمونی گریلین ل ناو خوین.

لە م توژینەوە (23) ئافرەت بەشداربوون کە تەمەنیان (٣,٦٤ ±٣,٨ سال) بوون و تووشی ئیشی نە رم بوونی ئیسك بوون , وئیشکەیان هاتبوونە دەست نیشان کردن ل ڕێگەی بکارئینانی ئامیری جووت أکس ل نەخوشخانەی ابن سینا فیرکردنی ل شاری موسل. هەموو نەخوشەکان دەرمانی الدرونیتان ورەگرت و ب دوزی 70 ملیگرام حەفتیانە و بو ماوەی (3) مانگ ل مانکی تشرینی دووەمی 2011 و ئاداری 2012. و هرموونی گریلین هاتیە شیکردنەوە بەری و پشتی بکارئینانی دەرمانی الدرونیت ب ریگای ئکارئینانی ئامیری الیزا.

دەرئانجام: ئەم توژینەوە دیار بوو بکارئینانی الدرونیت دەبیتە هوی زایدبوونی هرمونی گریلین ل خوینی مروۆ دا بە ڕێژەکەی بەر چاوو.

الخلاصة

تأثير عقار أقراص الدرونيت على مستوى الغريلين في مصل الدم للنساء في سن اليأس مصابات بهشاشة العظام

المقدمة: هشاشة العظام حالة مرضية تصيب الجهاز الهيكلي, تتميز بانخفاض كتلة العظم وتدهور البناء الهيكلي الدقيق لأنسجة العظام مما يؤدي إلى الزيادة في وهن العظم و يجعله أكثر عرضة للكسر. في الإنسان، تعد التغيرات التي تحدث في هرمون الاستروجين نتيجة التقدم في العمر وسن اليأس مصحوبا في انخفاض كتلة العظم المتزامن مع زيادة خطورة الأبة بالكسور. ويعد الغريلين هرمون معويا ببتيديا جديدا يتكون من (٢٨) حامض أميني تفرزه المعدة وتطلقه في حالة الصوم. ويؤدي هذا الهرمون دورا بارزا في التركيب العضوي للعظم وعملية اعادة بنائه.

هدف الدراسة: تهدف الدراسة الحالية الى تقييم تأثير عقار البسفوسفونيت (أقراص الدرونيت) المخفض لارتشاف العظم على مستوى الغريلين في مصل الدم للنساء في سن اليأس مصابات بهشاشة العظام.

المواد وطرائق العمل: شملت الدراسة (23) امرأة في سن اليأس متوسط أعمارهم (٣,٦٤ ±٣,٨ سنة) تم تشخيص هشاشة العظام بواسطة قياس كثافة العظم المعدنية باستخدام جهاز مقياس أشعة اكس مزدوج الطاقة (دكسا). أخذت العينات من مستشفى ابن سينا التعليمي في مدينة الموصل, حيث أعطي جميع المرضى عقار الالدرونيت وبجرعة (70) ملغم/أسبوعياً, وتم متابعتهم لفترة ثلاثة أشهر في الفترة التي تتراوح ما بين الأول من تشرين الثاني (2011) لغاية الأول من آذار (2012). تم قياس هرمون الغريلين في مصل الدم لجميع المرضى قبل وبعد العلاج بواسطة عدة تشخيصية خاصة بطريقة مقايسة الممتز المرتبط بالأنزيم (الاليزا).

النتائج: أظهرت الدراسة أن عقار الدرونيت المعطى لثلاثة أشهر أدى إلى زيادة معنوية في هرمون الغريلين في مصل الدم مع وجود علاقة عكسية بين مستوى هرمون الغريلين و مؤشر كتلة الجسم.

الاستنتاجات: استنتجت الدراسة الحالية ان عقار الألدرونيت قد ادى الى زيادة معنوية ملحوظة لمستوى الغريلين في مصل الدم لدى النساء.

THE EFFECT OF ZINC SUPPLEMENTATION ON DISEASE ACTIVITY IN PATIENTS WITH RHEUMATOID ARTHRITIS

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ABSTRACT

Background and objectives The levels of micronutrient especially zinc is significantly deteriorated in rheumatoid arthritis(RA), which in turn results in vicious cycle of damage and various grade of high Disease Activity Score derivative for 28 joints (DAS-28) in RA patients . The objective was to evaluate the effects of zinc supplementations on zinc status and disease activity in RA patients.
Methods: Ninety four patients (88 females and 6 males) participated in 6-months, intervention study. Baseline zinc status and biological markers of disease activity were measured and compared to that of 94 apparently healthy control subjects. Patients were assigned to receive 50 mg elemental zinc three times a day for six months period, as a complementary to their conventional drugs. Disease activity was assessed after intervention using the DAS-28.
Results: Significant change from baseline in DAS-28 was noted; high DAS-28 was seen in 25 patients (26.6%) after supplementation with zinc in contrast to 72 patients (76.6%) at the beginning of the study($p<0.01$) .Eight patients on zinc supplementation showed remission of disease (8.5%).
Conclusions: This study indicates that zinc supplementation play an important role in decreasing disease activity in these patients.

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Key words: Rheumatoid arthritis, zinc supplementation, disease activity

Rheumatoid arthritis (RA) is a chronic inflammatory, autoimmune disease resulting in joint inflammation. Rheumatoid arthritis has a worldwide distribution with an estimated prevalence of about 2% and prevalence increases with age, most dramatically in women with a peak appearing during middle age [1,2] . However the exact etiology of RA is still not elucidated to date, but the most commonly mentioned factors are: heredity, infectious agents and sexual factors [3]. Evidence suggested that the Reactive oxygen species (ROS) and oxidative stress are involved in pathogenesis of RA [4]. Epidemiological studies have shown that antioxidant system for removal of ROS is impaired in RA [5]. Trace elements including zinc are among contributing factors that have been considered to have a great role in the pathogenesis of RA [6]. Low levels of serum zinc have been previously reported in patients with rheumatoid arthritis in comparison to control healthy group [7] which may not be completely caused by low dietary zinc intake, Moreover low serum zinc might be caused by elevation in serum levels of some plasma cytokines in RA , such as Interleukin 1- α (IL-1-α) , Interleukin -6 (IL-6), and Tumor necrosis factor (TNF). These cytokines induce the synthesis of metallo- enzymes and thus, sequestration of Zn in the liver, pancreas and intestine, or by the use of corticosteroids and NSAIDs [8] . The role of zinc supplementation now a days is well-established and has been successfully used as a therapeutic and preventive agent for many conditions such as Wilson's disease,

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peptic ulcer , inflammatory acne , psoriasis, and several immune disorders [9,10,11,12,13]. But, in spite of several supplementation trials which were performed in patients with inflammatory conditions specially those suffering from rheumatic diseases , were insufficiently documented as far as the assessment of zinc status is concerned, making it difficult to correctly interpret the effects of zinc administration .Apart from some studies reported the beneficial effects of zinc supplementation with respect to joint swelling, morning stiffness and walking in patients with rheumatoid arthritis. So, to determine the effectiveness of zinc therapy on decreasing the clinical signs and symptoms of RA, this preliminary intervention study was designed to examine the effects of Zinc supplement on modification of disease activity score (DAS-28) in patients with RA.

METHODS

This Intervention study was conducted at the Duhok Center for Rheumatic Diseases and Medical Rehabilitation, Duhok, Kurdistan Region, Iraq; from January 2011 through March 2012.During a period of three successive months, 114 patients previously diagnosed with rheumatoid arthritis were participated in the study (106 registered cases routinely attended for follow-up and management and 8 cases were newly diagnosed as having Rheumatoid Arthritis). Ninety four patients were completed the trial (88 females and 6 males). The enrolment was done according to the revised 2010 criteria of American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) classification [14]. Ninety four apparently healthy control subjects selected from blood bank and kidney donors were included in the study. Patients were interviewed and informed about the nature of the study and then verbal consent was obtained from each subject. The study protocol was approved by the ethical Committee of the General Directorate of Health in Duhok Governorate

A pre-tested questionnaire was designed to obtain information, on age, gender, family history and duration of the disease. Other information included treatment and duration of treatment, number of tender and swelling joints, visual analog scale (VAS), and medical history of chronic diseases. Complete history and physical examination were done for all patients who were newly and previously diagnosed as RA. Baseline data and further monthly assessment were done for all participants at regular intervals, and finally complete assessment for recording of data after 6 months were done. Physical examination was performed in order to assess the severity and activity of the disease. The examination was included changes in previously affected joints or the appearance of inflammation in previously uninvolved joints. Examined joints include the wrists, elbows, shoulders, and knees, and the metacarpophalangeal and proximal interphalangeal joints of the hands. The joints were evaluated for the presence of swelling, tenderness, loss of motion and deformity. Disease activity was assessed by using the Disease Activity Score derivative for 28 joints (DAS-28) recommended by European League Against Rheumatism (EULAR): Low(DAS-28<3.2), Moderate (DAS-28 3.2to <5.1) and High activity(DAS> 5.1) A Protocol for zinc supplementation involved zinc sulphate capsule(220)mg three time daily which is equivalent to 50 mg elemental zinc (150 mg /day) for six months period, as a complementary to their conventional drugs(NSAIDs, Glucocorticoids, Methotrexate, Hydroxychlorquine and / or Aztioprine).Blood samples were collected after an overnight fast. After 25-30 mints, the serum was separated by centrifugation using a HITACHI centrifuge (model O5P-21) at 5000 rpm for 10 minutes at ambient temperature. The serum obtained was

separated and frozen at -20 C0 until the time of analysis. Urine samples were collected in polyethylene containers and centrifuged at 5000 rpm for 10 min. The supernatant obtained was separated and frozen at -20 C0 until the time of analysis.EDTA blood used for hematological parameters. Serum and urine Zinc levels were determined by using (Giesse Diagnostica-Italy)- kit in clinical chemistry analyzer Kinza 240.Inter- assay precision of method: Mean of 30 poled serum sample =89.12µg/dl ± SD= 4.41 mg/dl.Coefficients of variation (CV %) = (SD/mean) x100. CV%=4.94. Reference range : 70-120 µg/dl.Urine creatinine Was determined using Jaffe reaction method by clinical chemistry analyzer Kinza 240. EDTA blood samples were used for determination of hematological parameters(Hb and WBC) ,using automated hematological analyzer (Beckman coulters), and ESR.Rheumatoid Factor: (IgG,IgA,IgM) and High sensitivity C-Reactive Protein (hs-CRP):were determined by enzyme immunoassay kit (hs-CRP-ELISA) , from Biocheck, Inc ,catalog No.: BC-1119.Interleukin-6 (IL–6) levels in serum was measured by Assay Max human Interleukin-6 (IL-6) ELISA Kit catalog No. EI1006-1. Interleukin- 1 α (IL-1α) levels in serum was measured by Assay Max human Interleukin- 1 α (IL-1α) ELISA Kit catalog No. EI2301-1. Data were collected and analyzed using SPSS version 19.0 for windows (SPSS, Chicago; Illinosis, USA). Quantitative data were analyzed by using independent sample t-test. Qualitative data were analyzed by using chi-square test. Pearson's correlation coefficient was used to describe the association between the levels of zinc in serum and the related parameters.

RESULTS

(Table 1) shows the baseline zinc status in the RA patients and control subjects. Significant differences were observed regarding serum and urine zinc levels ($p<0.001$, $p<0.01$, respectively). The prevalence of hypozincaemia(serum zinc<70 ug/dl) was significantly higher among RA patients than controls (77.6%Vs 17.02%) . This finding was identical when the serum zinc levels adjusted to marginal and severe hypozincaemia.

Table 1.Zinc status in RA patients and Controls

Variables	RA patients	Controls	p-value
Serum Zinc(µg/dl)	*58.30±16.67	81.89±15.09	<0.001
Normozincaemia Serum Zn(>70ug/dl),n(%)	21 (22.34 %)	78 (82.98%)	<0.001
Marginal Hypozincaemia Serum(>50-70ug/dl), n(%)	34 (36.17 %)	16 (17.02 %)	< 0.01
Severe Hypozincaemia Serum Zn(<50ug/dl), n(%)	39 (41.49 %)	0 (0%)	<0.001
Urinary zinc excretion(Zinc/gm Creatinine)	*44.18 ±13.94	63.77± 13.40	<0.01

**Mean +SD,*

(Table 2) shows the effects of zinc supplementation on serum levels of zinc, inflammatory biomarkers and DAS-28 in RA patients. Meanwhile, serum zinc levels were significantly increased in RA patients ($p<0.001$) at the end of 6 months. DAS-28 values were statistically decreased after intervention ($p<0.001$). Inflammatory biomarkers (Rheumatoid factor, hs-CRP, Anti-CCP, IL-6 and IL1 α) reduction were significantly higher after 6 months supplementation($p<$ 0.01, for all

parameters).No significant differences were observed in regard to Hb and WBC. (Table 3) shows the distribution pattern of disease activity, zinc status, severity of disease, rheumatoid factor and anti-CCP cutoff points before and after 6months of

Table 2. Effects of zinc supplementation on disease activity in RA patients

Variables	Before	After	*P- value
DAS-28	5.76 ± 0.796	4.55 ± 1.02	< 0.01
NTJ	13.24 ± 5.32	10.19 ± 4.42	< 0.01
Zinc (µg/dl)	58.30 ± 16.68	95.94 ± 27.34	< 0.001
ESR (mm/hr)	48.15 ± 16.57	29.62 ± 12.76	< 0.001
Hb (g/dl)	12.13 ± 1.27	12.84 ± 1.46	NS
WBC	8.55 ± 1.63	9.12 ± 1.73	NS
VAS	69.25 ± 10.26	50.11 ± 12.49	<0.001
Rheumatoid factor (IU/ml)	127.05 ±118.37	101.71 ±101.00	<0.001
A-CCP (IU/ml)	26.65 ± 29.69	20.14 ± 13.17	<0.05
h-SCRP (mg/L)	24.25 ± 10.58	18.44 ± 9.60	< 0.01
IL-6 (pg/ml)	88.40 ± 34.87	76.94 ± 22.03	< 0.001
IL-1α (pg/ml)	48.52± 21.82	39.44 ± 17.84	< 0.001

Values are given as Mean+SD, NS: non-significant, p>0.05

Table 3 .Distribution pattern of disease activity, zinc status, severity of disease, rheumatoid factor and anti-CCP cutoff points before and after 6 months

Variables		Baseline Data		6 months		*P-value
		N	%	n	%	
DAS	Low DAS (≤ 3.2)	1	1.1	7	7.4	< 0.001
	Moderate DAS: 3.3≤ 5.1	21	22.3	54	57.4	
	High DAS > 5.1	72	76.6	25	26.6	
	Remission < 2.6	0	0	8	8.5	
Zinc level	Normozincaemia > 70 (µg/dl)	21	22.3	80	85.1	< 0.001
	Marginal Hypozicaemia: 50-70(µg/dl)	34	36.2	13	13.8	
	Severe Hypozicaemia < 70 (µg/dl)	39	41.5	1	1.1	
Severity of Disease	mild Disease Form	9	9.6	16	17.00	< 0.001
	Moderate Disease Form	36	38.3	56	59.6	
	Severe Disease form	49	52.1	22	23.4	
Rheumatoid Factor	Negative < 16	0	0	0	0	NS
	Equivocal : 16-24	1	98.9	4	4.3	
	positive > 24	93	1.1	90	95.7	
Anti-CCP	Negative < 15	24	25.5	30	31.9	NS
	Positive > 15.1	70	74.5	64	68.1	

** Chi test , NS:non-significant, p>0.05*

zinc supplementation. Disease activity as measured with DAS-28 was significantly improved, Moderate DAS-28 : 3.3≤ 5.1 was noted in 54 patients (57.4%) Vs 21 patients (22.3%) and remission of disease (DAS -28 < 2.6) was seen in 8 patients (8.5%). Severe disease form was seen in 22 patients (23.4%) in contrast to 49 patients (52.1%) at the baseline study. Additionally, there were significant changes in zinc status. No change was observed in the Rh factor and in the anti-CCP after 6 months of zinc supplementation.(Table 4) shows the values of Pearson's correlation coefficients between serum zinc levels and duration of disease, DAS-28 , ESR. Hb,WBC, and inflammatory biomarkers (Rheumatoid factor, hs-CRP, Anti-CCP, IL-6 and IL1 α). A negative correlation was observed between the serum zinc levels and the disease activity (Figure 1) determined by DAS-28(r= -0.648, p<0.001).Also, the serum zinc levels were negatively correlated with the inflammatory biomarkers, but the correlation was not significant with the duration of the disease (r =0.029, P=0.778).

Table 4. Pearson's correlation between the levels of zinc in serum and duration of disease,DAS-28, inflammatory factors and hematological indices in RA patients

Variables	R	P-value
Duration of disease	**0.029**	**NS**
DAS-28	**- 0.648**	**<0.001**
ESR (mm/hr)	**- 0.622**	**<0.001**
Hb (g/dl)	**-0.282**	**<0.01**
WBC	**-0.166**	**NS**
VAS (Cm)	**-0.530**	**<0.001**

NS: non-significant, p>0.05

DISCUSSION

In a view of the previous literatures, decreased plasma zinc levels have been reported in RA patients when compared with normal controls [15]. However marginal zinc deficiency appears to be an important public health problem in many developing countries including Iraq [16,17]. In our sample of subjects included, marginal zinc deficiencies do exist, even among controls. For example, a high prevalence of zinc deficiency, defined as serum zinc less than 70 µg/dl was demonstrated in this study among rheumatoid patients and healthy controls, which markedly decline from 77.66 % in rheumatoid to 17.03 % in controls. Thus a large group of rheumatoid patients may be at risk for developing zinc deficiency. It is noteworthy that 36.17 % appear at risk for marginal zinc deficiency and 41.49 % for severe zinc deficiency. Moreover there were statistically significant negative correlation between serum zinc levels and hematological, serological and DAS-28 .among RA patients (p < 0.001). The lower concentrations of serum zinc among RA patients might be contributed to several factors: inflammatory cytokines which causes shifting of zinc into peripheral tissues, drugs specially NSAIDs, steroids, in addition to decreased absorption of zinc, due to dietary habits that contain high phytates and finally lower zinc intake [18]. Our data show that subjects with RA had higher level of IL-6 and IL-1α than controls. These mediators induce the synthesis of metallothioenins and overburdening of Zn in the liver [19]. This, in

turn, results in serum Zn depletion in patients with active RA. Data from previous studies suggested a correlation between the extent of inflammation and serum Zn depletion [20].

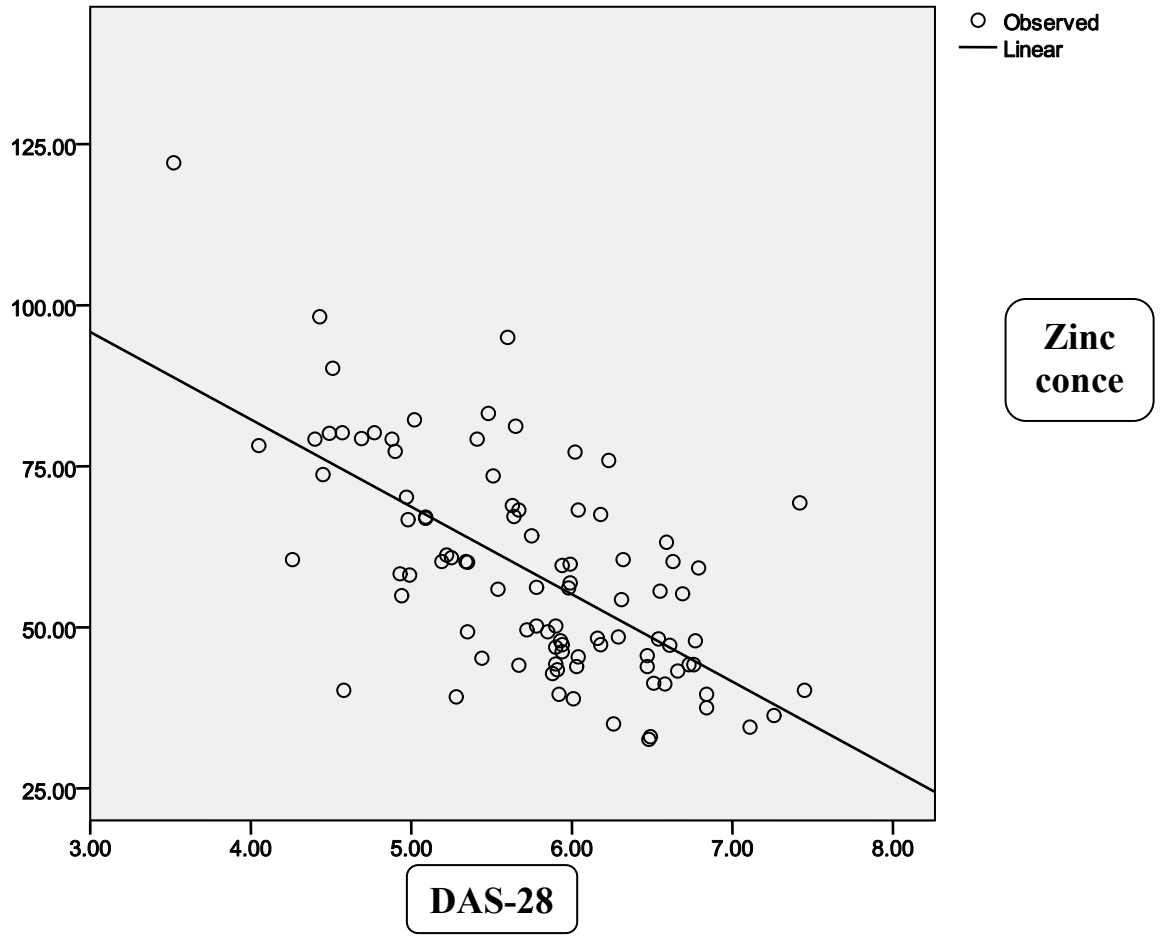

Figure 1 .The correlation between ser-+um zinc concentration and DAS-28

Although there have been some studies investigating the role of zinc in RA patients, there are poor data on role of zinc in pharmacotherapy and pathogenesis of RA in our community. The clinical value of Zinc supplementation in RA patients were first examined by Rasker JJ, Kardaun SH, the authors using 267 mg zinc sulphate doses (267 mg Zn as sulphate per day), in a preliminary trial encouraging results were obtained despite the fact that most of the patients were suffering from severe disease, refractory to conventional treatments [21]. Unfortunately, these results could not be confirmed by subsequent trials [22]. In our study, we designed a new zinc supplementation trial which included high dose of zinc form with known bioavailability and good tolerance for six months duration and as a complementary to their fixed regime of treatment (conventional drugs). On view of the well established anti-inflammatory and immunomodulating properties of zinc [23,24], zinc supplementation under the present conditions had produced notable beneficial measurable effects with the selected indicators of inflammatory status and disease activity.

As far as zinc status is concerned, our treatment resulted in significant modifications in plasma zinc, 85.1% of patients had increased in serum zinc levels, only 14.9 % show no increment in their

serum zinc level, which was consistent with previous reports [25]. The most interesting finding of this trial was zinc treated patients shows improvements in their DAS-28 and severity of disease. Finally a highly statistically significant differences after the zinc supplementation in ESR, Hb , IL-6, IL - 1 α. Anti-CCP and hs-CRP , were noted in 6 months treated patients , which might be explain that replenished zinc in RA patients might have anti-inflammatory value as reported previously [26].

CONCLUSION

Our results confirm the role of zinc in RA patients and indicate that zinc supplementation play an important role in decreasing disease activity in these patients. However, these findings suggest the need for designing more randomized controlled clinical trials to evaluate the impact of zinc therapy in different stage of disease activity in RA patients.

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پوختە

کارتێکرنا خارنا کەرەستێ زنکی ل سەر چالاکیا نەخوشیێ لدەف نەخوشێن روماتزمێ

پێشەکی و ئارمانج: رێژا خاردەمەنیێت هویر بتایبەت زنکی بشێوەیەکێ بەرجاڤ تێکدجیت لدەڤ نەخوشێن روماتزمێ ئەوا دبیتە ئەگەرێ بازنەکا ڤالا ژ زیانڤێکەتنێ و پلەییێن هەمەجور ژ چالاکیا نەخوشیێ یا بەرز ئەوا 28 گەهان دگریت و تێتە دیارکرن ب (DAS-28).

ئارمنج: ژ ڤێ ڤەکولینێ ئەو بو هەلسانگاندا کارتێکرنا دەرماندا بزنکی لسەر رێژا زنکی د خوینێ دا و هەر وەسا لسەر چالاکیا نەخوشیێ لدەف نەخوشێن روماتزمێ.

رێکێن کاری:ئەڤێ ڤەکولینێ 94 نەخوش بخوڤە گرتن" (88 ژ مێیا و6 ژ نێرا) ئەوێن پشکداری د ڤەکولینێ دا کرین بو ماوێ 6 مەهان رێژا هەرێك ژ زنکی و هەر وەسا دیارکەرێن بایولوجی دخوینێ دا وەك پیڤەرییێن دەست پێکی هاتنە پیڤان.هەر وەسا ئەڤ کەسە هاتنە هەمبەرکرن دگەل 94 کەسێن دیار کو دساخلەم.50 مغ ژ کەرەستێ زنکی هاتە دیارکرن وەك دەرمان روژێ 3جاران زێدەباری ئەو دەرمانێت ئەو دخون.باشان ئەو نەخوش هاتنە هەلسەنگاندن بشتی 6 مەهان ژ خارنا کەرەستێ زنکی بریکا کارئانینا (DAS-28).

ئەنجام:گوهرینێن بەرجاڤ هاتنە دیتن درێژا (DAS-28) یادەست پێکی , وەك یادیار (DAS-28) یابلند لدەف 25 ژنەخوشان دابو (26.6٪) پشتی 6 مەهان ژ خارنا کەرەستێ زنکی بەرانبەری 72 نەخوشان (76.6٪) لدەست پێکا ڤەکولینێ(P<0.01) و هەر وەسا 8 ژنەخوشان (DAS-28) زور دابەزی.

دەرئەنجام: ڤەکولینێ دیارکر کو خارنا کەرەستێ زنکی رولەکێ گرنگ یێ هەی د دابەزینا چالاکیا نەخوشیێ لدەف نەخوشێن روماتزمێ

الخلاصة

تأثير مكملات الزنك على نشاط المرض في المرضى الذين يعانون من التهاب المفاصل الروماتويدي

خلفية واهداف البحث: أن مستويات المغذيات الدقيقة خاصة" الزنك تتدهورت و بشكل ملحوظ لدى المرضى الذين يعانون من التهاب المفاصل الروماتويدي والتي تؤدي بدورها الى حلقة مفرغة من الضرر و الدرجات المختلفة من النشاط العالي للمرض الذي يصب 28 مفصلا" ويحدد ب (DAS-28).

الهدف: هو تقييم آثار العلاج بالزنك على حالة الزنك و كذلك نشاط المرض لدى مرضى التهاب المفاصل الروماتويدي.

طرق البحث: شملت الدراسة أربعة و تسعون مريضا" (88 من الإناث و6 من الذكور) الذين شاركوا لمدة 6 اشهر.تم قياس نسبة كلا" من الزنك والموءشرات البايولوجية في المصل كمقياس أساسي لحالة الزنك و كذلك لنشاط المرض على التوالي, تمت المقارنة مع 94 شخصا" من الذين يبدون اصحاء. عيين 50 مغ من عنصر الزنك ثالثة مرات في اليوم لمدة ستة أشهرللمرضى مكملا" لأدويتهم التقليدية. تم تقييم نشاط المرض بعد التدخل العلاجي باستخدام (DAS-28).

النتائج: لوحظت تغيرات هامة في نسب (DAS-28) الأساسية " " حيث ان (DAS-28) العالي شوهد في 25 مريضا (26.6٪) بعد العلاج بمكملات الزنك بالمقارنة إلى 72 مريضا (76.6٪) في بداية الدراسة ($P<0.01$)وكذلك اظهرت أن ثمانية (8.5٪) من المرضى الذين كانوا على مكملات الزنك انخفضت (DAS-28) الى وضعية الركود.

الاستنتاج :اشارت هذه الدراسة أن العلاج بمكملات الزنك يلعب دورا هاما في خفض نشاط المرض في هؤلاء المرضى.

THE EFFECT OF METHANOL EXTRACTS OF SOME PLANTS AGAINST THE BACTERIA CAUSING DIARRHEA AMONG CHILDREN

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ABSTRACT

Five hundred samples of stool specimen were collected from patients with diarrhea (infants and children under ten years of age) admitted to the Pediatric and Maternity Hospital in Erbil City from March to September 2007. The samples were cultured on different culture media. The isolated bacteria were identify according to their colony morphology, biochemical reactions and by use of API 20E systems. 35(7%) *E. coli*. Biotype I, 8(1.6%) *E. coli* Biotype II, 17(3.4%) *E. coli* Biotype III, 22(4.4%) *E. coli* Biotype IV, 8(1.6%) *Shigella dysenteriae*, 16(3.2%) *Salmonella arizonae*, 12 (2.4%) *Salmonella typhi* and 6 (1.2%) *Vibrio cholera* were isolated.
Sensitivity test showed high resistance to amoxicillin/ clavulanic acid, amoxicillin, ampicillin, cefixime, erythromycin, nitrofurantoin, rifampicin, streptomycin, tetracycline, trimethoprim - sulfamethoxazole and tobramycin. Most of the isolated appeared sensitive to nalidixic acid, doxycyclin, gentamycin , cefotaxime , cephalexine , chloramphenicol and amikacin. All isolates of bacteria were sensitive to ciprofloxacin.
The MIC of the methanol extracts of *Prunus armeniaca* was ranged between 1.25000- 10.0000 mg/ ml and the MBC was 2.5000 - 20.0000 mg/ ml . While, the MIC of *Prosopis farcta* extract ranging 1.2500 - 20.0000 mg/ ml and the MBC was 2.5000 - 20.0000mg/ ml, the MIC of *Juglans regia* leave extract found to be 2.5000 - 20.0000 mg/ ml and the MBC was 5.0000 - 20.0000mg/ ml, and MIC of Juglans regia exocarpes extract was 1.2500 - 20.0000mg/ ml and the MBC was 2.5000 -20.0000 mg/ ml.

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Key words: Diarrhea, Plant extraction.

Diarrhea is best defined as excessive loss of fluid and electrolytes in the stool and should continue for a minimum of 2 weeks, before it is considered chronic. In infants, stool volume in excess of 15g/Kg/24hr. is considered diarrhea [34]. Diarrhea is also usually defined by parent as a consistent increase in stool frequency. Decrease in stool consistency, or increase in stool volume and frequency. Stool volume is extremely difficult to assess in infant because of the difficulty in separating stool from urine [19].

Bacterial infections are very important causes of diarrhea in infants and young children worldwide [20]. The principal microorganisms implicated are *Salmonella* [32], *Shigella* [30], *Vibrio cholerae* [33] and serotypes of *Escherichia coli* including EPEC, STEC, EAEC, EIEC and EHEC [26].

Microorganisms have developed resistance to many antibiotics and as a result, immense clinical problem in the treatment of infectious diseases has been created. The resistance of the organisms increased due to indiscriminate use of commercial antimicrobial drugs commonly used for the treatment of infectious disease [28]. This situation forced the researchers to search for new antimicrobial substances from various sources including medicinal plants [3,23].

The use of alternative medical therapy has increased the interest of pharmacologists and herbalists over the past decades. There is increasing use of herbal products all over the world, in USA, it reached 38% between 1990 and 1997 [7]; therefore, instead of antibiotic therapy, there is a continuing search for new antimicrobials,

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from other sources including plant extracts.

The fruits of *Prunus armeniaca* are anti diarrheal, antipyretic, emetic, allaying thirst and not good for old people. The seeds are tonic and anthelmintic, used in liver troubles, piles, ear ache and deafness. In Afghanistan, the fruits are considered to be laxative and refrigerant in fever [24]. The juice extracted from fresh apricot leaves can help treat scabies, eczema, sunburn and itching. Apricot has tonic, laxative and aphrodisiac properties. It helps fight infection, repair damaged tissue and develop strong teeth. In addition, it has high mineral content making it a good choice for treating anemia, tuberculosis and toxemia. Dye can be obtained from the leaves, which are considered to be astringent [16].

Walnut leaves are astringent, tonic and anthelmintic. The leaves and bark are alterative and detergent , given against herpes, eczema, scrofula and syphilis. Vinegar of the pickled young fruit is used as gargle in sore throat. The green hull and unripe shell are considered useful in syphilis and are vermin fungal. Walnut leaves selectively inhibited the growth of gram- positive and gram- negative , and fungi [10].

The present study was carried out to determined the antimicrobial activity of *Juglans regia* (Leaves), exocarps (Derum) and *Prunus armeniaca* (Leaves), against enteric bacteria.

METHODS

Sample collection

Five hundred stool samples were collected from infants and children (below ten years of age) suffering from diarrhea admitted to the Pediatric and Maternity Hospital in Erbil City, from March to September 2007.

Isolation and Identification of bacteria:-

Identification was done on the basis of morphology, cultural characteristics and biochemical tests. Then confirmed by using api20E system [4].

Preparation of crude extracts:-

Prunus armeniaca (leaves), *Juglans regia* (leaves and exocarp) and *Prosopis farcta* (pods) were used in present study, which classified by Dr. Abdul-Hussein Al-Khayat, College of Education, Salah Al-Deen University. The medicinal plants included in this study were washed with tap water and then dried.

For the preparation of Methanol crude extracts samples were prepared according to [13].

Determination of MIC Of antibiotic

To study the effect of different antimicrobials on all isolates of bacteria, Mueller-Hinton agar were used as growth media, after sterilization and cooling at 45°C, final concentration of antibiotics was added to media and poured into sterile Petri dishes. After solidification, the plates were inoculated by streaking method with bacterial isolates then incubated at 37°C for 24 hours. The results were recorded next day [18].

Determination of MIC and MBC values

The antibacterial activity of the extracts was determined by evaluating the MIC using the Micro Broth dilution method, serial dilutions of the extract were prepared (20mg/ml to 0.0195 mg/ml) directly into nutrient broth in microtiter. The wells were seed culture at final inoculums of 1E6 bacteria/ ml. The experiment was performed in triplicate. The bacterial suspensions were used as positive control and extracts in broth were used as negative control. Then the plate was covered with a sterile plate sealer. Contents of each were mixed on plate shaker at 300 rpm for 20 seconds and then incubated at 37 °C for 24 hours. Microbial growth was determined at 600nm using the ELX800 universal microplate reader. The results were recorded in the form of MIC

defined as the minimum amount of the extract that inhibit the growth of the microorganisms. While the least concentration showing no visible growth on agar subculture was considered as MBC value [8].

Study the inhibitory effect of different concentration of plant extraction:-

The extracts were dissolved in sterile distilled water to a final concentration 50mg/ml. The disc diffusion method was used to evaluate the antibacterial activity. Mueller Hinton agar was prepared in the plates as the media for the test microorganisms. Sterile filter paper discs were impregnated with 100 µl of each of the extracts, placed on Mueller Hinton agar plate inoculated w bacteria, then incubated for 24 hr. at 37° C. Distilled water served as negative control and ciprofloxacin was used as standard to confirm that all the microorganisms tested were inhibited by the antibiotic. The antibacterial activity was evaluated by measured the zone of the inhibition against the tested isolate [22].

RESULTS

The results showed 35(7%) *E. coli*. I, 8(1.6%) *E. coli* II, 17(3.4%) E. coli III, 22(4.4%) *E. coli* IV, 8 (1.6%) *Shigella dysenteriae*, 16(3.2%) *Salmonella arizonae*, 12 (2.4%) *Salmonella typhi* and 6(1.2%) *Vibrio cholera*.

The susceptibilities of (124) isolates were tested against (19) widely used antimicrobials. The *E. coli*. I showed resistance to chloramphenicol (95%), ampicillin(90%), amoxicillin/ clavulanic acid (86%), amoxicillin (80%), tetracycline (60%), streptomycin (56%), trimethoprim-sulfamethoxazole (50%), erythromycin (44%), cephalexine (36%), rifampicin (32%), cefixime (30%), cefotaxime (30%), doxycyclin (26%), gentamycin (20%), nitrofurantoin (9%) , nalidixic acid (9%) and tobramycin (8%) . All isolates of bacteria were sensitive to ciprofloxacin and amikacin (100%) each of them .

The *E. coli*. II showed resistance to amoxicillin (92%), chloramphenicol (90%), ampicillin (87%), amoxicillin/ clavulanic acid (85%), tetracycline (65%), streptomycin (50%), trimethoprim-sulfamethoxazole (45%) ,erythromycin (40%), cefixime (39%), cephalexine (39%), rifampicin (30%), cefotaxime (30%), doxycyclin (25%), gentamycin (18%), tobramycin (10%) , nalidixic acid (9.5%) and nitrofurantoin (8%) . All isolates of bacteria were sensitive to ciprofloxacin and amikacin (100%) each of them.

The *E. coli.* III showed resistance to amoxicillin/ clavulanic acid (90%), ampicillin (89%) amoxicillin (88%), chloramphenicol(80%), tetracycline (70%), streptomycin(52%), trimethoprim-sulfamethoxazole (45%), erythromycin (42%), cefixime (35%), cefotaxime (35%), cephalexine (35%), rifampicin (29%), doxycyclin (28%), gentamycin (18%), nalidixic acid (10%) , tobramycin (8.5%) and nitrofurantoin (8.5%). All isolates of bacteria were sensitive to ciprofloxacin and amikacin (100%) each of them.

The *E. coli.* IV showed resistance to amoxicillin/ clavulanic acid (89%), ampicillin(89%), chloramphenicol (85%), amoxicillin (81%), tetracycline (66%), streptomycin (55%), trimethoprim-sulfamethoxazole (55%), erythromycin (43%), cefixime (34%), cefotaxime (33%), rifampicin (30%), cephalexine (30%), doxycyclin (22%), gentamycin (22%), nitrofurantoin (9.8%), nalidixic acid(9.5%) and tobramycin (8%). All isolates of bacteria were sensitive to ciprofloxacin and amikacin (100%) each of them. (Figure 1 a).

The *Salmonella arizonae* showed resistance to streptomycin (100%), nalidixic acid (100%), erythromycin (100%), cephalexine (100%), amoxicillin (90%), amoxicillin/ clavulanic acid (80%), tetracycline (80%), gentamycin (80%), ampicillin (70%), cefixime (70%),

tobramycin (70%), rifampicin (50%), trimethoprim-sulfamethoxazole (30%), amikacin (20%), cefotaxime (20%). All isolates of bacteria were sensitive (100%) to ciprofloxacin, nitrofurantoin, doxycyclin , and chloramphenicol .

The *Salmonella typhi* showed resistance to amoxicillin (100%), erythromycin (100%), cephalexine (100%), nalidixic acid (100%), tobramycin (80%), chloramphenicol (80%), ampicillin(80%), streptomycin (80%), amoxicillin/ clavulanic acid (75%), rifampicin(70%), tetracycline (70%), trimethoprim-sulfamethoxazole (70%), gentamycin (60%), cefixime (30%), amikacin (25%), cefotaxime (20%) and doxycyclin (5%). All isolates of bacteria were sensitive (100%) to ciprofloxacin and nitrofurantoin, (Figure 1 b).

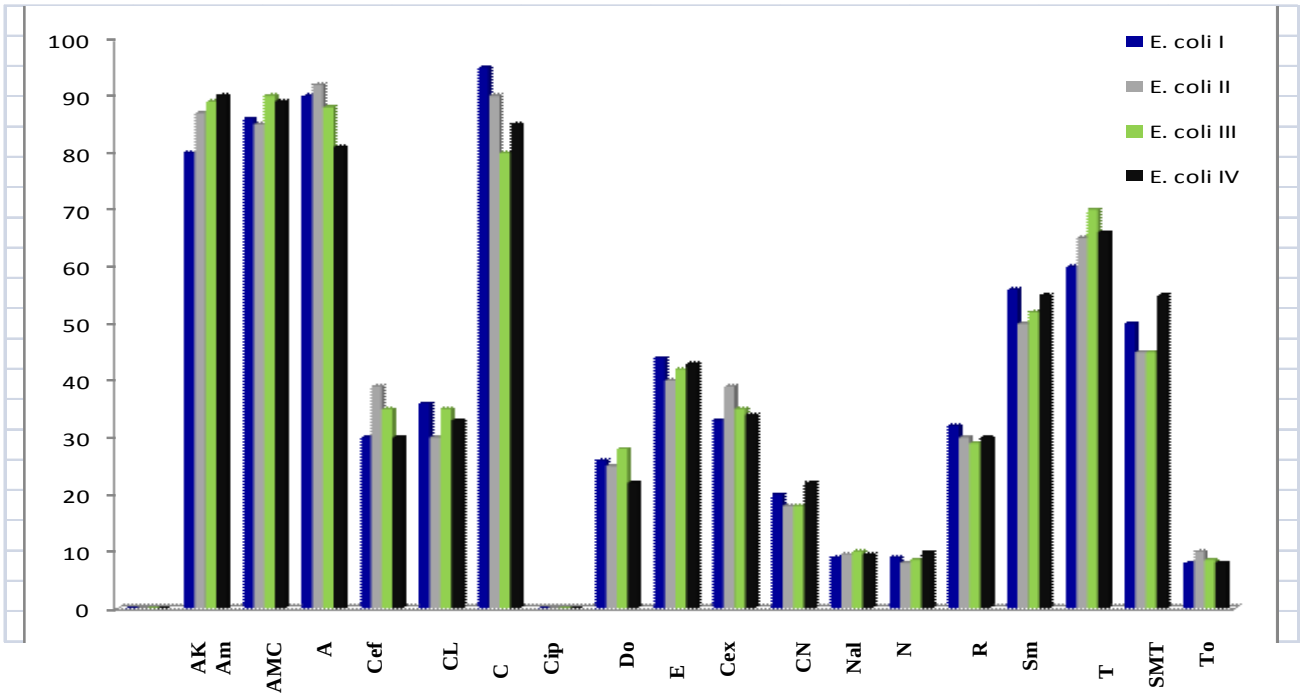

Figure 1a. Percentage of susceptibility pattern of *E. coli* in standard antibigram

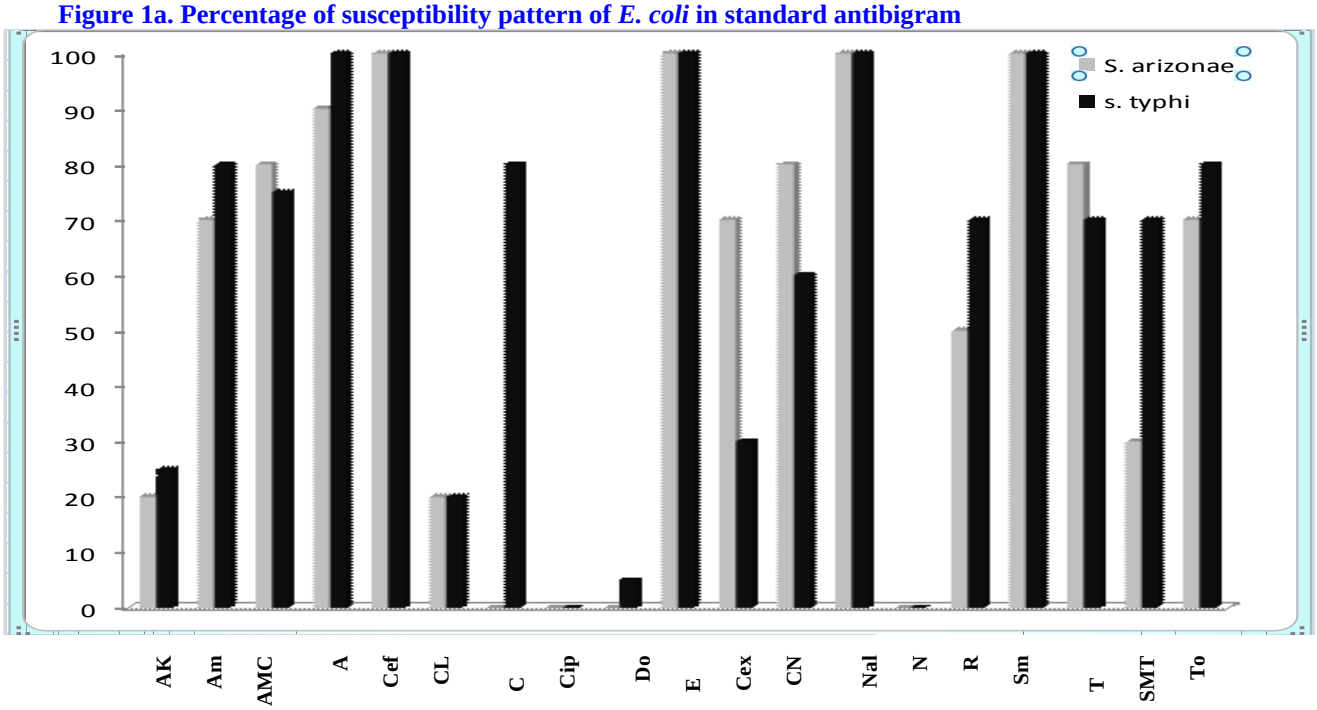

Figure 1b. Percentage of susceptibility pattern of *Salmonella spp*. in standard antibigram.

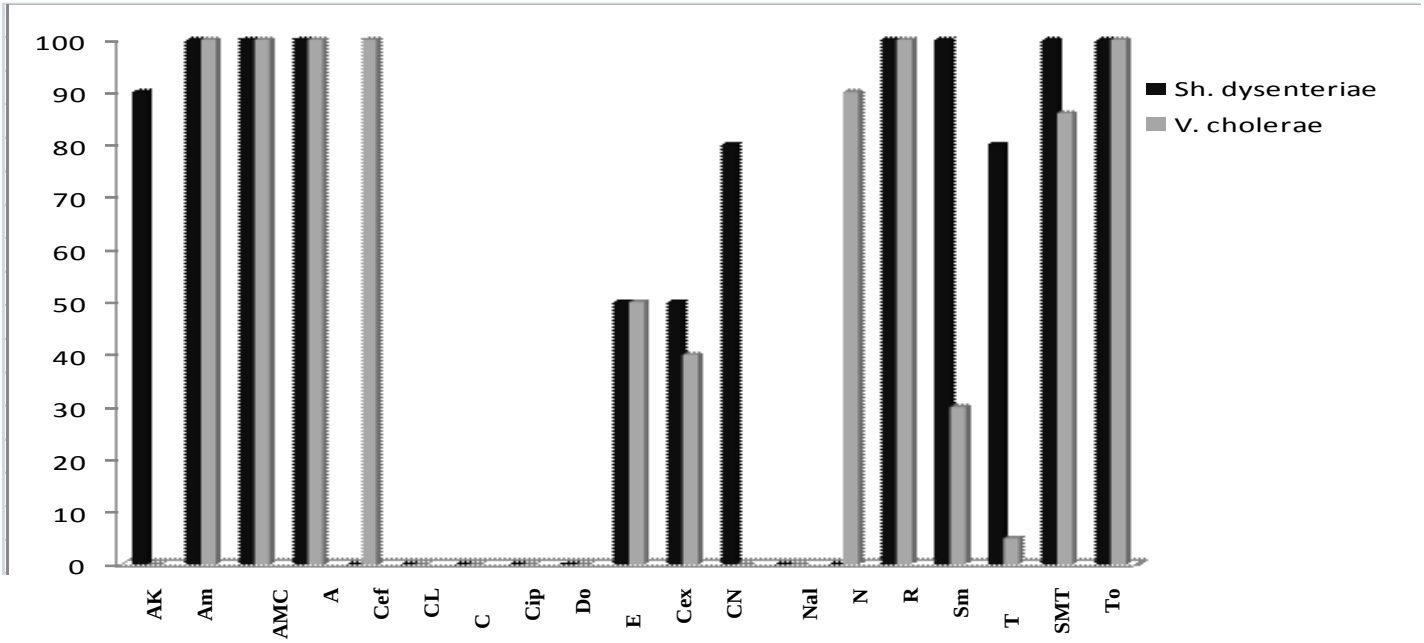

Figure 1C. Percentage of susceptibility pattern of *Shigella* and *Vibrio* in standard antibigra

***Amoxicillin/clavulanic acid(AMC), Amoxicillin(AM), Ampicillin(A), Cefixime(Cex), Erythromycin(E), Nitrofurantoin(N), Rifampicin(R), Streptomycin (Sm) , Tetracycline (T), Trimethoprim–Sulfamethoxazole (S) (T), Tobramycin (T), Nalidixic acid (Nal), Doxycyclin (Do), Gentamycin(CN), Cefotaxime (Cef), Cephalexine (CL), Chloramphenicol (C), Amikacin (Ak) and Ciprofloxacin(Cip).*

The *Shigella dysenteriae* showed resistance to amoxicillin/ clavulanic acid (100%), amoxicillin (100%), ampicillin (100%), streptomycin (100%), amikacin (90%), tetracycline (80%), gentamycin (80%), tobramycin (70%), cefixime (50%), erythromycin (50%), rifampicin (50%) and trimethoprim-sulfamethoxazole (30%). All isolates of bacteria were sensitive (100%) to ciprofloxacin, nalidixic acid, cefotaxime, cephalexine, doxycyclin, nitrofurantoin and chloramphenicol

The *Vibrio cholerae* showed resistance to amoxicillin/ clavulanic acid (100%), amoxicillin (100%), ampicillin (100%), rifampicin (100%), cephalexine (100%), tobramycin (100%), trimethoprim-sulfamethoxazole (86%), erythromycin (50%), cefixime (40%), streptomycin (30%) and tetracycline (5%). All isolates of bacteria were sensitive (100%) to ciprofloxacin, nalidixic acid, amikacin, gentamycin, cefotaxime, doxycyclin, nitrofurantoin and chloramphenicol (Figure 1 c). Antibacterial activity of plant extracts against isolates showed that crude Methanolic extract of *J. regia* leaves no effect against *E.coli* polyvalent II, *Shigella desenterae, S. arizonae, S. typhi* and *V. cholerae*. But more active against *E. coli* polyvalent I, III, IV result in inhibition zones of 1 mm -15mm, 1mm -9mm and 5mm -9mm respectively (Table 1).

Methanolic extract of *J. regia* exocarps against bacterial strains of *E. coli* I, II, III, IV produced inhibition zones 5mm-15mm, 10mm -13mm, 10mm-15mm and 9mm -15 mm, respectively. While against *Sh. desenterae* 7 mm, *S. arizona* 3mm-8 mm, *S. typhi* 2mm-6 mm and *V. cholera* 6mm-30 mm (Table 2).

The result of antibacterial activity of Methanolic extract of *P. armeniaca* leaves against *E. coli* I, II, III, IV, *Sh. desenterae, S. arizonae, S. typhi* and *V. cholera* revealed that the inhibition zones 5mm-20mm, 5mm-25mm, 10mm-20mm , 5mm-10mm, 6mm-15mm, 6mm-9mm, 7mm-15mm and 0.5mm-0.9mm respectively (Table 3).

Table 1. Antibacterial activity of Methanol extraction of *Juglans regia* leaves at different concentration against bacteria

Vibrio cholerae	*Salmonella typhi*	*Salmonella Arizona*	*Shigella desenterae*	*E.coli polyvalent IV*	*E.coli polyvalent III*	*E.coli polyvalent II*	*E.coli polyvalent I*	Concentration (mg/ml)	No.
Diameter of zone inhibition (mm)									
0	0	0	0	9	1	0	15	20.0000	1
0	0	0	0	8	9	0	1	10.0000	2
0	0	0	0	7	5	0	9	5.0000	3
0	0	0	0	5	0	0	8	2.5000	4
0	0	0	0	0	0	0	6	1.2500	5
0	0	0	0	0	0	0	0	0.6250	6
0	0	0	0	0	0	0	0	0.1563	7
0	0	0	0	0	0	0	0	0.0781	8
0	0	0	0	0	0	0	0	0.0391	9
0	0	0	0	0	0	0	0	0.0195	10
15	14	14	15	15	15	15	15	Ciprofloxacin	C

** Inhibition zone (in mm)*
C: control antibiotic

Table 2. Antibacterial activity of Methanol extraction of *Juglans regia* exocarps at different concentration against bacteria

Vibrio cholerae	*Salmonella typhi*	*Salmonella Arizona*	*Shigella desenterae*	*E.coli polyvalent IV*	*E.coli polyvalent III*	*E.coli polyvalent II*	*E.coli polyvalent I*	Concentration (mg/ml)	No.
Diameter of zone inhibition (mm)									
30	6	8	7	15	15	13	15	20.0000	1
25	2	3	0	10	10	10	10	10.0000	2
20	0	0	0	9	0	10	9	5.0000	3
10	0	0	0	0	0	0	7	2.5000	4
6	0	0	0	0	0	0	5	1.2500	5
0	0	0	0	0	0	0	0	0.6250	6
0	0	0	0	0	0	0	0	0.1563	7
0	0	0	0	0	0	0	0	0.0781	8
0	0	0	0	0	0	0	0	0.0391	9
0	0	0	0	0	0	0	0	0.0195	10
15	14	14	15	15	15	15	15	Ciprofloxacin	C

** Inhibition zone (in mm)*
C: control antibiotic

The results of antibacterial activity of Methanolic extract of *P. farcta* against *E. coli* I, II, III, IV and *Sh. desenterae* , revealed that the inhibition zones 5mm-15mm, 10mm-30mm, 5mm-8mm, 5mm-6mm and 5mm-8mm respectively. There were no effects of this extraction against *S. arizonae, S. typhi* and *V. cholera* (Table 4). All inhibition zones were compared with ciprofloxacin as a control, where the sensitive strains displayed an inhibition zone more than 21mm, intermediate

resistance, 16mm-20mm and the resistant strains less than 15 mm in diameter.

Table 3. Antibacterial activity of Methanol extraction of *Prunus armeniaca* leaves at different concentration against bacteria

Vibrio cholerae	*Salmonella typhi*	*Salmonella Arizona*	*Shigella desenterae*	*E.coli polyvalent IV*	*E.coli polyvalent III*	*E.coli polyvalent II*	*E.coli polyvalent I*	*Concentration (mg/ml)*	No.
Diameter of zone inhibition (mm)									
0.9	15	9	15	10	20	25	20	20.0000	1
0.5	7	6	10	5	15	20	18	10.0000	2
0	0	0	8	0	10	18	16	5.0000	3
0	0	0	6	0	0	15	11	2.5000	4
0	0	0	0	0	0	10	5	1.2500	5
0	0	0	0	0	0	5	0	0.6250	6
0	0	0	0	0	0	0	0	0.1563	7
0	0	0	0	0	0	0	0	0.0781	8
0	0	0	0	0	0	0	0	0.0391	9
0	0	0	0	0	0	0	0	0.0195	10
15	14	14	15	15	15	15	15	Ciprofloxacin	C

** Inhibition zone (in mm)*
C: control antibiotic

Table 4. Antibacterial activity of Methanol extraction of *Prosopis farcta* at different concentration against bacteria

Vibrio cholerae	*Salmonella typhi*	*Salmonella Arizona*	*Shigella desenterae*	*E. coli polyvalent IV*	*E. coli polyvalent III*	*E. coli polyvalent II*	*E. coli polyvalent I*	Concentration (mg/ml)	No.
Diameter of zone inhibition (mm)									
0	0	0	8	6	8	30	15	20.0000	1
0	0	0	6	5	5	25	8	10.0000	2
0	0	0	5	0	0	13	5	5.0000	3
0	0	0	0	0	0	11	0	2.5000	4
0	0	0	0	0	0	10	0	1.2500	5
0	0	0	0	0	0	0	0	0.6250	6
0	0	0	0	0	0	0	0	0.1563	7
0	0	0	0	0	0	0	0	0.0781	8
0	0	0	0	0	0	0	0	0.0391	9
0	0	0	0	0	0	0	0	0.0195	10
15	14	14	15	15	15	15	15	Ciprofloxacin	C

** Inhibition zone (in mm) C: control antibiotic*

Table 5. Determination of MIC and MBC values of methanol extraction of *Juglans regia* leaves against different bacteria.

Vibrio cholerae	*Salmonella typhi*	*Salmonella Arizona*	*Shigella desenterae*	*E.coli polyvalent IV*	*E.coli polyvalent III*	*E.coli polyvalent II*	*E.coli polyvalent I*	Concentration (mg/ml)
0.144	0.124	0.101	0.122	0.015**	0.004	0.154**	0.030**	20.0000
0.189**	0.210**	0.241**	0.154**	0.020*	0.005**	0.189*	0.140*	10.0000
0.201*	0.255*	0.235*	0.233*	0.021	0.141*	0.221	0.231	5.0000
0.255	0.247	0.354	0.278	0.011	0.185	0.344	0.268	2.5000
0.298	0.311	0.410	0.344	0.102	0.221	0.387	0.310	1.2500
0.341	0.356	0.456	0.450	0.221	0.275	0.462	0.375	0.6250
0.365	0.378	0.510	0.487	0.322	0.341	0.487	0.421	0.1563
0.388	0.401	0.522	0.465	0.366	0.451	0.520	0.475	0.0781
0.401	0.421	0.433	0.501	0.451	0.495	0.571	0.514	0.0391
0.489	0.520	0.520	0.521	0.411	0.514	0.582	0.520	0.0195
0.501	0.566	0.586	0.578	0.574	0.557	0.601	0.589	Control

**MIC ** MBC*

Table 6. Determination of MIC and MBC values of methanol extraction of *Juglans regia* exocarpes against different bacteria.

Vibrio cholerae	*Salmonella typhi*	*Salmonella Arizona*	*Shigella desenterae*	*E. coli polyvalent IV*	*E. coli polyvalent III*	*E. coli polyvalent II*	*E. coli polyvalent I*	Concentration (mg/ml)
0.001	0.001	0.022**	0.010*	0.014	0.012**	0.010	0.001	20.0000
0.002	0.015**	0.015*	0.120	0.015**	0.011*	0.010**	0.001	10.0000
0.002**	0.014*	0.145	0.175	0.010*	0.102	0.011*	0.002	5.0000
0.012	0.178	0.185	0.204	0.145	0.175	0.104	0.020 **	2.5000
0.010*	0.159	0.245	0.268	0.175	0.254	0.175	0.021*	1.2500
0.174	0.159	0.286	0.321	0.205	0.310	0.254	0.102	0.6250
0.186	0.201	0.305	0.387	0.287	0.388	0.296	0.178	0.1563
0.254	0.254	0.386	0.402	0.387	0.402	0.304	0.254	0.0781
0.301	0.366	0.401	0.462	0.399	0.475	0.386	0.266	0.0391
0.399	0.366	0.421	0.501	0.402	0.499	0.425	0.347	0.0195
0.602	0.425	0.522	0.633	0.520	0.601	0.522	0.586	Control

** MIC ** MBC*

The MIC of the methanol extracts of *Juglans regia* leave ranging between 2.5000 mg/ml - 20.0000 mg / ml and the MBC was 5.0000 mg/ml - 20.0000mg/ ml (Table 5). The MIC of the *Juglans regia* exocarpes extract found to be 1.2500 mg / ml - 20.0000 mg/ ml and the MBC was 2.5000 mg / ml -20.0000 mg/ ml (Table 6). The MIC of *Prunus armeniaca* extract was between 1.25000 mg / ml - 10.0000 mg/ ml and the MBC was 2.5000 mg / ml - 20.0000 mg/ ml (Table 7). In addition, the MIC of the methanol extracts of *Prosopis farcta* was between 1.2500 mg / ml - 20.0000 mg / ml and the MBC was 2.5000 mg / ml -20.0000 mg/ ml (Table 8)

Table 7. Determination of MIC and MBC values of methanol extraction of *Prunus aremeniaca* leaves against different bacteria.

Vibrio cholerae	*Salmonella typhi*	*Salmonella Arizona*	*Shigella desenterae*	*E. coli polyvalent IV*	*E. coli polyvalent III*	*E. coli polyvalent II*	*E. coli polyvalent I*	Concentration (mg/ml)
0.010**	0.001**	0.031**	0.001	0.001**	0.002	0.005	0.004	20.0000
0.015*	0.007*	0.030*	0.002	0.021*	0.010**	0.004	0.002	10.0000
0.122	0.201	0.174	0.001**	0.122	0.015*	0.001	0.002**	5.0000
0.210	0.245	0.245	0.024*	0.154	0.154	0.020**	0.022	2.5000
0.254	0.310	0.286	0.154	0.254	0.185	0.015*	0.020*	1.2500
0.354	0.386	0.354	0.254	0.268	0.254	0.020	0.123	0.6250
0.412	0.423	0.362	0.278	0.312	0.301	0.175	0.175	0.1563
0.521	0.456	0.425	0.341	0.401	0.356	0.301	0.254	0.0781
0.555	0.478	0.451	0.301	0.456	0.421	0.342	0.354	0.0391
0.601	0.498	0.511	0.421	0.574	0.486	0.425	0.421	0.0195
0.675	0.591	0.582	0.541	0.962	0.501	0.489	0.541	Control

* *MIC* ** *MBC*

Table 8. Determination of MIC and MBC values of methanol extraction of *Propspis farcta* against different bacteria.

Vibrio cholerae	*Salmonella typhi*	*Salmonella Arizona*	*Shigella desenterae*	*E. coli polyvalent IV*	*E. coli polyvalent III*	*E. coli polyvalent II*	*E. coli polyvalent I*	Concentration (mg/ml)
0.211	0.120	0.004*	0.012	0.010*	0.023**	0.012	0.010**	20.0000
0.341	0.154	0.145	0.015**	0.140	0.022*	0.010	0.021*	10.0000
0.389	0.186	0.240	0.025*	0.140	0.231	0.010	0.104	5.0000
0.390	0.210	0.298	0.140	0.254	0.255	0.015**	0.186	2.5000
0.451	0.254	0.344	0.139	0.366	0.366	0.024*	0.254	1.2500
0.521	0.288	0.397	0.254	0.388	0.421	0.120	0.269	0.6250
0.522	0.310	0.414	0.286	0.452	0.489	0.222	0.351	0.1563
0.532	0.389	0.452	0.354	0.562	0.499	0.362	0.398	0.0781
0.544	0.451	0.472	0.421	0.565	0.502	0.487	0.452	0.0391
0.581	0.480	0.498	0.475	0.564	0.515	0.498	0.486	0.0195
0.581	0.560	0.560	0.501	0.584	0.560	0.560	0.501	Control

**MIC*
*** MBC*

DISCUSSION

In this study, bacterial pathogen were found in (24.8%) out of the all the samples, 35(7%) *E.coli*. I, 8(1.6%) *E. coli* II, 17(3.4%) *E. coli* III, 22(4.4%) *E. coli* IV, 8(1.6%) *Shigella dysenteriae*, 16(3.2%) *Salmonella arizonae*, 12(2.4%) *Salmonella typhi* and 6 (1.2%) *Vibrio cholera*. These results were agree with Brad et al. [6] who found pathogenic *E. coli* and *Salmonella spp*. were (1.81%) each of them. These results were agree with the result of Behiry et al[5] who found that pathogenic *E. coli* was the most common isolate group.

Most biotype of *E. coli* were highly resistant to amoxicillin (80-90%), amoxicillin / clavulanic acid (85-90%) and ampicillin (81-92%). All isolates of biotype *E. coli* were sensitive to cephalexine and ciprofloxacin this is similar to which was obtained by Estrada-Garcia et al[11], who found all isolates

susceptible to ciprofloxacin and cefotaxime. However, ciprofloxacin and other quinolones are not approved for children because of the risk of damage to immature joints and most parental third generation cephalosporins (cefotaxime) are administered only in a hospital setting.

Shigella spp. seems to be resistance to ampcilline, amoxicillin and rifampicine There are similar results to those who detected by Al-Shuwalli [1] .*Shigella spp*. was found to be highly sensitive to ciprofloxacin, nalidixic acid and cefotaxime Hawezy [14].

All *Salmonella spp*. were sensitive to aminoglycosides, Beta lactam, qiunolones, co- trimoxazole group and azithromycin [29]. The resistance of *V. cholera* to trimethoprim-sulfamethoxazole and erythromycin were (86%) and (50%) respectively, Similar results were recorded by Keramat et al[15] where there was a (98%) and (62%) resistance for trimethoprim- sulfamethoxazole and erythromycin respectively .*V. cholera* was also found to be susceptible to cephalexine, nalidixic acid, gentamycin and fluroquinolones [2] this is already agrees with our results. Several resistance mechanisms such as plasmid encoded resistance, mutation in the quinolones resistance determine regions, intergrons and efflux pumps are responsible for this resistance [12].

The methanolic extracts of *Juglans regia* leave showed inhibitory effects 1mm-15 mm, where, the zone of inhibition was observed against *E. coli* I 15mm.

The inhibition zones effect of methanolic extracts of *Juglans regia* exocarpes against all E. coli strains were in range 5mm-15 mm. While against *Shigella dysentery, S. arizonae, S. typhi* and *V. cholera* were 7 mm, 3mm-8 mm, 2mm-6 mm and 6mm-30 mm, respectively. The MIC of the methanol extracts of *Juglans regia* exocarpes was 1.2500 mg/ ml - 20.0000 mg/ ml and the MBC was 2.5000 mg/ ml - 20.0000 mg/ ml. This result competitive with Sharafati –Chaleshtori et al. [27] who show the minimum inhibitory concentrations (MIC) for extract of *Juglans regia* ranged between 15.6 mg/ ml and 187.5 mg/ml and minimum bactericidal concentrations (MBC) ranged between 31.25 mg/ ml and 250 mg /ml. These results may be due to the absence of tannin compound in alcoholic extracts of Juglans regia leaves. Astringent tannins are important ingredients of walnut leaves and these tannins cross-link with the skin cells enabling them to be resistant to allergies and diseases caused by microorganisms. It is also used to help control diarrhea and menorrhagia. In addition, walnut contains quinones, oils, phenolic acids which have antioxidant, anticarcinogen, antibacterial and antiviral activities [21,31].

The inhibition zones effect of methanol extracts of *Prunus armeniaca* against *E. coli* I, *E. coli* II, *E. coli* III, *E.coli* IV , *Shigella dysentery*, *S. arizonae , S . typhi* and *V. cholera* were 5mm-20mm, 5mm-25 mm, 10mm-20mm, 5mm-10mm, 6mm-15mm, 6mm-9mm, 7mm-15mm and 0.5mm-0.9mm respectively. The MIC of the methanol extracts of *Prunus armeniaca* was between 1.25000 mg/ ml - 10.0000 mg/ ml and the MBC was 2.5000 mg/ ml - 20.0000 mg/ ml. This result is comparable to that of Rashid [25] who reported that the MIC values of Gram-positive bacteria were 31.25 mg/ ml -250 mg/ml and 125 mg/ ml -250 mg/ml for Gram-negative bacteria except Enter pathogen *E. coli* which had an MIC value of 500 mg/ml. Different parts of the plant are used for the treatment of many ailments, mainly against diseases of bacterial and fungal origins. In the Yunani system of medicine, it is used as an antidiarrheatic, emetic and anthelmintic in lever diseases, piles, earache and deafness, lung diseases and abscesses [25].

In addition, The effect of methanol extracts of *Prosopis farcta* against *E. coli* I, *E. coli* II, *E. coli* III, *E.coli* IV and *Shigella dysentery* and *S. arizonae* were 5mm-15mm, 10mm-30mm, 5mm-8mm, 5mm-6mm and 5mm-8mm respectively.

There are no effects of the extraction against *S. arizonae, S. typhi* and *V. cholera.* The MIC of the methanol extracts of *Prosopis farcta* was between 1.2500 mg/ ml - 20.0000 mg / ml and the MBC was 2.5000 mg/ ml-20.0000 mg/ ml. This result is comparable to that of Mahasneh et al. [17] who reported that the inhibition zone of *E. coli was* between (7-12) mm. Darwish [9] reported the MIC and MBC values of extracts of *Prosopis farcta* against *S. flexneri* was 100 mg/ml. Mahasneh et al. [17] reported petroleum , ether, methanol, Hexane, butanol and aqueous crude extracts of the whole aerial parts of *Prosopis farcta* and others exhibited variable degrees of antimicrobial activity against *E. coli* , Streptococcus spp. and Acinetobacter spp. bacterial and two fungal species compared with that exerted by antibiotics.

CONCLUSIONS

The isolated revealed high resistance to most widely used antibiotic, the extraction of the medical plant showed high potential an antibacterial agent.

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پوختە

كاريگەريا ئاڤا پوختەيا ميپانولێ يا هندەك رووەك وداروبارا دژى بەكتريا بێت كو زك چوونێ پەيدا دكەن دناڤ زاروكادا.

پێنج سەد نمونێت پيساتى يێ بچيكا هاتە كومكرن ژ وان نەخوشا بێت توش زك چوونێ بوين (زاروك وبچيكێت دبن ژيێ دەھ ساليێ دا) بێت كو هاتينە نڤاندن ل خەستەخانا زاروكا وبچيك بوونێ لباژێرێ هەولێرێ ژ هەيڤا ئادارا 2007 هەتا هەيڤا 9 يا 2007 ئەڤ نمونێت پيساتى هاتنە چاندن دناڤ ناڤەندێت جياجيادا بو پشكنينێ.

ئەو بەكتريايێت هاتينە ڤەدەركرن هاتنە نياسين ل گور مورفولوجيا وان، كارلێكێت كيميائى وب كارئينانا سيستەمێ (API 20 E) 35 ژ وان (7 ٪) *E. Coli* ژ جورێ I ، 8 ژ وان (1.6 ٪) *E. Coli* ژ جورێ II ، 17 ژ وان (3 و 4 ٪) *E. Coli* ژ جورێ III ، 22 ژ وان ئانكو (4.4 ٪) *E. Coli* ژ جورێ IV ، 8 ژ وان (1.6 ٪) ديار بون كو *Shigell adysenteriae* ، 16 ژ وان (3.2 ٪) *Salmonella Arizonae* ، 12 ژ وان (2.4 ٪) *Salmonella Typhi* و 6 بێت مايى ئانكو (1.2 ٪) ديار بوون كو *Vibrio Cholera* بوون.

پشكنينێت هەستاريێ ديا ركرن كو بەرگريەكا بلند هەبوو بەرامبەر اموكسيسيلين ، كلاڤولانيك أسيد، أموكسيسيلين، أمبيسيلين، سيفيكسين، أريپرومايسين، نيتروفيورانتين، ريفامبيسين، ستربتومايسين، تيتراسايكلين، تريميپوبرين ، سلفاميپوكسازول، و توبرامايسين.

بارا پترى بەكتريايێت هاتينە ڤەدەركرن ديار بوون كو هەست دارن بو ڤان دەرمانان ناليديكسيت أسيد، دوكسى سايكلين، جينتامايسين، سيفوتاكسيم، سيفالكسين، كلورامفيليكون، وأميكايسين. هەمى بەكتريايێت هاتينە ڤەدەركرن د هەست دار بوون بو سيبتروفلوكساسين.

نزمترين چرپا داشكاندى يا ئاڤا پوختەيا ميپانولێ يا خورنيفكا *Prounus Armeniaca* دناڤبەرا 1.25000 – 10.000 ملغم/مل و نزمترين چرپا كوژەك دناڤبەرا 2.5000 – 20.4000 ملغم/مل. بەلێ پا نزمترين چرپا داشكاندى يا مشمژا *Prosupis Farcta* دناڤبەرا 1.2500 – 20.0000 ملغم/مل ونزمترين چرپا كوژەك يا وى دناڤبەرا 2.5000 – 20.000 ملغم/مل، هەر چەندە نزمترين چرپا داشكاندى يا بەلگێت گيزا ديار بوو كو 2.5000 – 20.000 ملغم /مل و نزمترين چرپا كوژەك يا وێ 5.0000 – 20.0000 ملغم/مل، و نزمترين چرپا داشكاندى يا ئاڤا تيفكلێ گيزێ 1.2500 – 20.0000 ملغم/مل و نزمترين چرپا كوژەك يا وێ دناڤبەينا 2.5000 – 20.0000 ملغم/مل.

الخلاصة

دراسة التاثير المثبط لبعض المستخلصات النباتية الميثانولية ضد انواع مختلفة من البكتريا المسببة للاسهال لدى الاطفال

تم جمع 500 عينه خروج اطفال يعانون من الاسهال (اطفال حديثي الولادة واطفال دون سن العاشرة من العمر) والمراجعين لمستشفى الاطفال والولادة في محافظة اربيل للفترة اذار 2007 الى ايلول 2007. زرعت النماذج على أوساط زرعية مختلفة و إعتمادا على الصفات المزرعية ، الشكلية و التفاعلات الكيموحيوية وإستخدام نظام ال API 20E تم تشخيص 35 (7٪) *E. coli*. I ، 8 (1.6٪) *E. coli* II ، 17 (3.4٪) *E. coli* III ، 22 (4.4٪) *E. coli* IV ، 8 (1.6٪) *Shigella dysenteriae* ، 16 (3.2٪) *Salmonella arizonae* ، 12 (2.4٪) *Salmonella typhi* ، 6 (1.2٪) *Vibrio cholera* .

اظهرت جميع العزلات البكتيرية قيد الدراسة مقاومة عالية لكل من مضادات الحياتية الاموكسسلين/حامض كلافيولانك ، الاموكسسلين ، الامبسلين ، النتروفولنين ، الارثرومايسين ، سيفاكسيم ، ريفامبسين، ستربيومايسين، تتراسايكلين ، تريمثبريم – سلفوميثازول والتوبرومايسين ، في حين اظهرت اغلب العزلات حساسية عالية لكل من مضادات الحياتية حامض النالدكسك ، ديوكسيسايكلين ، الجنتاميسين ، الاميكاسين ، الكلورومافنكول ، السيفاتكسيم والسيفالكسيم ، وكانت جميع العزلات البكتيرية قيد الدراسة حساسة لمضاد السبروفلوكساسين.

حددت التراكيز المثبطة الدنيا (MIC) والتراكيزالقاتلة الدنيا (MBC) ومناطق التثبيط لاربع نباتات (اوراق الجوز ، قشرة الجوز ، الخرنوب والمشمش) على عزلات E.coli *I*، *E.coli II*، *E.coli III E.coli IV* ، *Shigella dysenteriae* ، *Salmonella arizonae* ، *Salmonella typhi* و *Vibrio cholerae*. وكانت قيمة التركيزالمثبط الادنى للمستخلص الميثانولي لنبات الخرنوب يتراوح بين 1.25000-10.0000 ملغم / مل ، اما التركيزالمثبط القاتل الادنى كان يتراوح بين 2.5000 – 20.0000 ملغم / مل ، في حين كان التركيز المثبط الادنى للمستخلص الميثانولي لنبات المشمش يتراوح بين 1.2500– 20.0000 ملغم /مل والتركيز المثبط القاتل الادنى يتراوح بين 2.5000– 20.0000 ملغم / مل ، اما بالنسبة للمستخلص الميثانولي لمستخلص اوراق الجوز فقد كان التركيز المثبط الادنى له يتراوح بين 2.5000–20.0000 ملغم/مل والتركيز القاتل الادنى 5.0000– 20.0000 ملغم / مل ، واظهرت التركيز المثبطة الدنيا للمستخلص الميثانولي لقشره الجوز من 1.2500 الى 20.0000 ملغم / مل والتركيز القاتل الادنى له يتراوح بين 2.5000 –20.0000 ملغم /مل .

A CYTOPATHOLOGICAL STUDY OF THE EFFECT OF SMOKING ON THE ORAL EPITHELIAL CELLS IN RELATION TO ORAL HEALTH STATUS BY THE MICRONUCLEUS ASSAY

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ABSTRACT

Background: Micronucleus is a cytoplasmic fragment of DNA reported as a biomarker of cancer. It is a cytoplasmic chromatin mass formed in the basal cells layer of the epithelium. These fragments can form their own membrane. The aims of the study was to detect the micronuclei expression in the oral epithelial cells in cytopathological smears of the non-smokers' and the smokers' males, correlate the micronuclei expression in the oral epithelial cells with the oral health status variables, and evaluate the efficacy of the micronuclei assay to detect the subjects at high risk of oral mutations.
Methods: This study was conducted on 75 males of (35- 40) years of age divided into 25 heavy smokers, 25 light smokers, and 25 non-smokers. A cytobrush was used to obtain the smears. The oral health status was evaluated by using the plaque, gingival, calculus indices in addition to the amalgam and composite restorations.
Results: There was a statistically significant difference in the micronuclei expression among the three groups. There was a strong correlation between the oral health status variables and the micronuclei expression in the non- smokers' group, for the Plaque index with (P-value =0.0005) and for the calculus index (P-value = 0.04). The smokers' group had a strong correlation with the amalgam restorations with (P-value =0.0005).
Conclusion: The micronucleus assay detected by Pap stain is a useful biomarker to detect the people at high risk of oral mutations due to the harmful effect of the smoking, the calculus and plaque indices, in addition to the amalgam restorations.

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Key words: Cytopathology, micronucleus assay, smoking effects.

The oral epithelial cells represent the preferred target site for the early genotoxic events induced by different types of agents entering the oral cavity [1]. The smoking is a complex mixture of different type substances that are with a genotoxic and a carcinogenic effect on the oral epithelial cells. These substances lead to the DNA damage and the nuclear anomalies formation. One of these a nuclear anomaly is the micronuclei formation [2].

Micronucleus is a cytoplasmic fragment of DNA reported as a biomarker of mutagenesis. It is a cytoplasmic chromatin masses that can form its own membrane. The micronuclei are formed in the basal cells layer. The micronucleus assay is used to detect the subjects at high risk of malignant transformations in their oral epithelial cells [3]. It has been extensively used to evaluate the extent of chromosomal damage in the human population exposed to the genotoxic agents in various occupational settings, in the environment, or as a consequence of the life style [4].

The micronuclei test is gaining an increased attention among researchers and laboratories in the field of environmental mutagenesis, and a number of published studies based on this biomarker are increasing rapidly [5].

Oral cytopathology is a simple technique that is non-aggressive, relatively painless, and readily accepted by the patient. It is used to obtain cells from the oral epithelium. It is the art and science of interpretation of the cells obtained from the oral cavity. The oral epithelial cells

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may be detached naturally or artificially as in scrubbing and the cytobrush sampling [6]. In the current study, micronuclei assay was sought to be used for the first time on Iraqi sample to evaluate its validity as a biomarker for the early detection of the oral epithelial cells with mutation in relation to the effect of the smoking as a very popular habit, on the oral epithelial cells from different keratinized and non-keratinized oral sites. The smoking is a complex mixture of different types of substances that have a mutagenic and carcinogenic effect on the oral epithelial cells. The smoking effect on the oral epithelial cells was evaluated in relation to the different oral health status variables. The oral health status variables include the plaque, gingival, calculus indices and the amalgam and the composite restorations.

METHODS

Seventy five males' volunteers aged (35-40) year attending to the oral diagnosis department/collage of dentistry/Baghdad University, the maxillofacial clinic in Al Hussein hospital, and specialized center for Dentistry in Karbala. The oral examination includes the determination of the types of restorations in each tooth by the application of the FS fraction of the DMFS index. Oral health status for each patient was assessed by using plaque, gingival and calculus index.

Oral smear should be obtained from normal mucosa by using the cytopathological brush. The oral sites include: - buccal mucosa, hard palate, gingiva and floor of mouth. The smears were transferred and spread onto the labeled, clean, dry glass slide. Each slide was labeled with the patient's name ,the site from which the sample obtained and the type of stain .For Pap stain method , the slides were fixed at once by 95% ethanol for 20 minutes, whereas, they were air dried for Giemsa stain method. Each oral site has two slides, the first one was stained by Pap stain and the second by Giemsa stain. Each patient has 8 slides and 1000 oral epithelial cells examined by the light microscope.

RESULTS

All the subjects of the study sample were with a positive expression of the micronuclei in different numbers (Tables 1,2, and 3) (Figures1,2,3, and 4). There was a statistically significant difference in the micronuclei expression between the non-smokers and the light smokers, where (P= 0.031) in the floor of mouth stained by Pap stain. There was a statistically significant difference in the micronuclei expression between the non- smokers and the heavy smokers where (P = 0.0005) in both the floor of mouth and the gingiva, (P = 0.002) in the buccal mucosa, and (P= 0.004) in the palate stained by Pap stain. For the slides stained by Giemsa stain, floor of mouth, gingiva, and the buccal mucosa (P = 0.001), while palate was a non-significant difference (P=0.685). There was a statistically significant difference between the light smokers and the heavy smokers where (P = 0.0005) for all the oral sites stained by Pap stain, and floor of mouth stained by Giemsa stain only showed a highly significant difference (P=0.0005). Both gingiva and palate were with a (P=0.521), while buccal mucosa was (P = 0.59) for the slides stained by Giemsa stain for the micronuclei expression (Table4). There was a strong correlation between the oral health status variables and the micronuclei expression in the non- smokers' group, for the Plaque index with (P =0.0005) and for the calculus index (P = 0.04). Regarding the smokers' group, they had a strong correlation with the amalgam restorations with (P =0.0005). According to the multiple linear regression model indicated that the non-smokers' group was with (P =0.007) for the calculus index, while in the smokers' group (P = 0.006) for the amalgam restorations

Table 1.Comparison of demographic characteristic between induction group and conservative group.

No.	Variables	Induction N =60	Conservative N=60	P. value
1	Age (years)*	29.72±4.3	26.95±5.4	0.482
2	Primi gravid	29 (48. 33%)	26 (43.33%)	0.120
3	Multigravida	31 (51. 66%)	34 (56.66%)	0.05
4	Previous post term	10 (16%)	16 (26%)	0.000

** results are expressed as mean ±standard deviation P value of less than 0.05 is of statistical significance*

Table 2 .Comparison of oxytocin augmentation between induction group and conservative group.

No	Maternal outcomes	Induction No. and % 60 (100%)	Conservative No. and % 60 (100%)	P. value
1	Oxytocin used	25 (41.66)	34 (56.7)	0.103
	Oxytocin not used	35 (58.33)	26 (43.3)	0.103
2	Mode of delivery			
A	Vaginal delivery	50 (83.33)	28 (46.66)	0.00
B	Cesarean section delivery	10 (16.66)	32 (53.33)	0.00
	Maternal satisfaction			
3	Yes	54 (90)	35 (58.33)	0.00
	No	6 (10)	25 (41.66)	0.00
4	Post partum hemorrhage	2 (3.33)	3 (5)	0.64

** P value of less than 0.05 is of statistical significance*

Table 3. Comparison of indication of cesarean between induction and conservative groups.

No.	Parameter	Induction No. and % 60 (100%)	Conservative No. and % 60 (100%)	P. value
1	Failure of progress of labor	5(8.3%)	7(11.6%)	0.062
2	Fetal distress	2(3.6%)	23(38.3%)	0.000
3	Prolonged second stage of labor	3(5%)	2(3.6%)	0.114

**P value of less than 0.05 is of statistical significance*

Table 4. Comparison of fetal outcomes between induction group and conservative group.

No.	Parameter	Induction No. and % 60 (100%)	Conservative No. and % 60 (100%)	P. value
1	Meconium stain liquor			
	Thin meconium	10 (16.6)	18 (30)	0.000
	Thick meconium	2 (3.33)	12 (20)	
2	Apgar score			
	<7 at 5 minutes	3 (5)	10 (16.7)	0.015
3	Admition in to NCU	2 (3.33)	16 (26)	0.000
4	Weight	3.6±0.44	3.6±0.36	0.438
5	Emergency c/s for fetal distress	2 (3.3)	23 (38.33)	0.000
6	Sex of infant			
	Female	26 (43.33)	34 (56.66)	0.144
	Male	34 (56.66)	26 (43.33)	

** P value of less than 0.05 is of statistical significance*

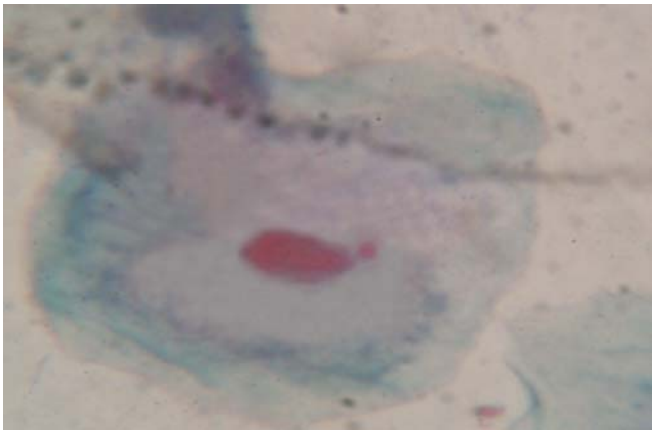

Figure1: An oral epithelial cell with micronucleus stained by Pap stain at X100 oil emersion.

Figure 2: An oral epithelial cell with micronucleus stained by Giemsa stain at X100 oil emersion.

Figure3: An oral epithelial cell with micronucleus stained by Pap stain at X40.

Figure4: An oral epithelial cell with micronucleus stained by Giemsa stain at X40

DISCUSSION

The study results revealed that, there was a micronuclei expression in all the smears taken from the examined males, but in different proportions. The mean of the micronuclei expression in the non-smokers' group was (2.36) micronucleus in each 1000 oral epithelial cells. This was consistent with the baseline of the micronuclei expression in the healthy subjects which was (0.5-2.5) micronucleus per 1000 oral epithelial cells according to the results of[1] who studied the micronuclei expression in the oral epithelial cells from patients with cancer, pre-cancerous lesions, and healthy controls. They found an 11 fold increase in the micronuclei expression in the patients with cancerous oral lesions (p-value = 0.001) and (10.38) fold increase in the micronuclei expression in the patients with pre-cancerous oral lesions (p-value =0.002). The current study was also related to the oral health status variables and their effect on the oral epithelial cells in relation to the smoking by the micronuclei expression. In the non-smokers' group, there was a strong relation of the plaque index and the calculus index with the increase in the micronuclei expression in the oral epithelial cells since the dental plaque and calculus represent sites for the oral bacteria which produce the chronic bacterial infection, the chronic infection is usually lead to the chronic inflammatory process that is often associated with the human carcinogenesis and the formation of clastogenic and anuploid genetic damage in the oral epithelial cells [8]. So these indices have a prominent effect on the oral epithelial cells by increasing the rate of micronuclei. The micronuclei expression in the non-smokers resulted from the effect of the plaque and calculus indices in addition to the effect of the environmental pollutants and the passive smoking or the spicy and hot food.

In the heavy smokers' group and according to the results statistically analyzed especially on the buccal mucosa, there was a strong effect of the amalgam restorations in relation to the smoking on the micronuclei expression in the oral epithelial cells which could be attributed to that the heavy smoking will inhibit the growth of the oral bacteria and enhance the growth of tar resistant bacteria on the oral epithelial cells that have a carcinogenic effect by increase the micronuclei expression. Additional to the path of the poisonous effect of smoking on the buccal mucosa, the unfavorable effect of the amalgam restorations on the oral epithelial cells due to the direct contact of the amalgam restorations on the oral epithelial cells and the metallic ions released from these restorations. The biological interaction of the restorations with the oral epithelial cells is related to the toxic and allergic reactions and the increase in the bacterial adherence which can lead to the inflammatory effects [9].

CONCLUSION

The micronucleus assay detected by Pap stain is a useful biomarker to detect the people at high risk of oral mutations due to the harmful effect of the smoking, the calculus and plaque indices, in addition to the amalgam restorations.

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پوختە

ڤەکولینا سایتوباتولوجی یی تأسیرا جگەرەکێشانی ل سەر خانێن دەڤ ل مروڤی بەروار دگەل ساخلەمیا دەڤ ب ڕێکا بکارئینانا ناوک بچیک (micronucleus)

ئامانج لە توێژینەوە: ناوک بچیک (micronucleus) پارچا سایتوپلازمی یە یاDNA و هاتیە نیاسین وەک نیشانا بایولوجی یا شێرپەنجەی . مەبەس ل ڤی ڤەکولینی دەست نیشان کرنا ناوک بچیک ل خانێن دەڤ ل سمیرا سایتوباتولوجی یا جگەرەکێشا و جگەرانەکێشا.

ڕێکێن ڤەکولینی: 75 نێر هاتینە دەستنیشان کرن بو ئەنجامدانا ئەڤ ڤەکولینە و هاتینە دابەش کرن ل سەر 3 گروپا, 25گەلەک جگارا کێشن و25 کێم جگارا کێشن و25 جگارا نا کێشن. وcytobrush هاتیە بکارئینان بو دەستڤەئینانا سمیرا. وساخلەمیا دەڤ هاتیە هەلسەنگاندن ب ڕێکا بکارئینانا amalgam and composite , plaque, gingival, calculus indices restorations.

ئەنجام: دیار بو کە پیوەندی ئاماری ب هێز هەیە دناڤبەرا هەرسێ گروپا,

There was a strong correlation between the oral health status variables and the micronuclei expression in the non- smokers' group, for the Plaque index with (P-value =0.0005) and for the calculus index (P-value = 0.04). The smokers' group had a strong correlation with the amalgam restorations with (P-value =0.0005).

دەرئەنجام: ناوک بچیک ئەوی هاتیە بویاغکرن ب بویاغا پاپ نیشانا بایولەجی ب مفایا بو دەسنیشان کرنا مروڤێن ل مەترسی شەرپەنجا دەڤ ل جگەرەکێشا دیسان بو دەسنیشان کرناamalgam and composite , plaque, gingival, calculus indices restoration

الخلاصة

دراسة باثولوجي خلوي من تأثير التدخين على الخلايا الظهارية الفموية فيما يتعلق بالوضع الصحي عن طريق الفم بواسطة الفحص النواة الصغرى

خلفية: النواة الصغيرة هو جزء من الحمض النووي حشوية الابلاغ عن العلامات البيولوجية لسرطان. بل هو كتلة ونين حشوية شكلت في طبقة الخلايا القاعدية من البشرة. ويمكن لهذه الشظايا تشكيل غشاء الخاصة بهم. كانت أهداف الدراسة للكشف عن التعبير النويات في الخلايا الظهارية الفموية في مسحات باثولوجي خلوي من غير المدخنين والمدخنين الذكور، ربط التعبير النويات في الخلايا الظهارية الفموية مع متغيرات الوضع الصحي عن طريق الفم، وتقييم فعالية للمقايسة النويات للكشف عن المواد المعرضة لمخاطر عالية من الطفرات عن طريق الفم.

المواد والطرق: أجريت هذه الدراسة على 75 من الذكور (35 – 40) سنة من العمر وتنقسم الى 25 المدخنين الشرهين، 25 يدخنون، و 25 من غير المدخنين. تم استخدام cytobrush الحصول على مسحات. تم تقييم الحالة الصحية عن طريق الفم باستخدام لوحة، لثوي، ومؤشرات حساب التفاضل والتكامل، بالإضافة إلى مزيج مركب والترميم.

النتائج: كان هناك فروق ذات دلالة إحصائية في التعبير النويات بين المجموعات الثلاث. كان هناك وجود علاقة قوية بين المتغيرات حالة صحة الفم والتعبير النويات في المجموعة غير المدخنين، للمؤشر البلاك مع (ف = 0.0005 القيمة) وللمؤشر حساب التفاضل والتكامل (ف القيمة = 0.04). وكان مجموعة من المدخنين وجود علاقة قوية مع الترميم الملغم مع (ف = 0.0005 القيمة).

الخلاصة: إن مقايسة النواة الصغرى الكشف عنها بواسطة صبغة باب هو علامة بايولوجية مفيدة للكشف عن الأشخاص المعرضين لخطر عال من الطفرات عن طريق الفم بسبب التأثير الضار للتدخين، المؤشرات حساب التفاضل والتكامل والبلاك، بالإضافة إلى الترميم الملغم.