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CONTENTS

EPIDEMIOLOGY OF INTESTINAL PARASITES AMONG FOODHANDLERS IN ERBIL CITY

Abbas Mohamed Faraj, PhD. *
Qaraman Mamakhidr Koyee , MSc. **

Submitted 28 Apr 2012; accepted 31 May2012

ABSTRACT

Background and Objectives: An epidemiological prospective study was carried out during the period from beginning of October 2005 to the end of March 2006 to investigate the types and prevalence of intestinal parasites among the foodhandlers in Erbil governorate.
Methods: A total of 587 stool samples were collected from foodhandlers of 10≥50 years old age groups. The stool samples were examined by direct and concentration methods (floatation and sedimentation). Zinc-sulfate, sugar and salt solutions used in floatation, while formalin-ether used for sedimentation.
Results and Conclusions: The total rate of infection among foodhandlers was 26.58% of which 24.53% were protozoans and 2.21% were helminthes. Zinc-sulfate flotation method showed higher efficiency in diagnosing intestinal parasites, followed by saturated salt solution flotation method. The differences among these methods were statistically significant at both levels of 0.01 and 0.05.
The survey among foodhandlers showed the presence of the following parasites: among protozoans *Entamoeba histolytica* with the higher incidence of (12.95%), followed by *Giardia lamblia* 8.006*%, Entamoeba coli* 3.24% and the least infection percentage was 0.17% for both *Iodamoeba buetschlii* and *Trichomonas hominis*. Among helminthes infections are *Hymenolepis nana* in a high percentage of 2.04% and the lowest incidence of 0.17% for *Ascaris lambricoides* were recorded. Single parasitic infection was the highest with a percentage of 21.29%, double infection 2.55% and only one case of triple infection in the rate 0.17 % of the total infection was recorded. No significant difference was noticed for different age groups; however the few differences noticed were not statistically significant.

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Key words: Intestine; Parasites; Foodhandlers; Erbil City.

Human intestinal parasitic infections are spread, most commonly, through the ingestion of food or water contaminated with human or animal feces as a result of poor sanitation. Parasitic infections have a worldwide distribution, occurring in developing and developed countries, but the heaviest burden of disease occurs in the developing world [1].
In general, many studies have been done on intestinal parasites by using zinc-sulfate flotation and formaline-ether sedimentation. Truant et al. [2] have examined 280 fecal specimens by different concentration methods, 50 positive specimens were identified. Only slight differences in the detection of parasites were found for the methods. The recovery of *E.coli, G. lamblia* cysts and *H. nana* ova was slightly better with the zinc sulfate procedure, but the detection of Taenia and Ascaris ova was somewhat better with the sedimentation procedures.
Molan and Farag[3] have examined fecal samples of schoolchildren from two different districts of Erbil-City (Koran and Azadi). Out of the total number of samples (424), the rate of infection was 62.74%, the rate of infection for Koran district was higher (77.585) than that of Azadi district 46.27%, they recorded *G. lamblia, E. histolytica, E. coli, H. nana* and *A. lumbricoides* in the rate 35.59%, 18.6%, 48.5%, 28.32% and 19.72% respectively for Koran district, while for Azadi district

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the rates were 27.20%, 6.93%, 26.27%, 14.57% and 10.12% respectively.

Al-Kachache[4] indicated the incidence of intestinal parasites in some villages of Nineveh province, in which several hundred stool samples were collected, examined by both direct and zinc-sulphate floatation methods. The percent of infection was 40.7%, which includes both protozoa (19.14%) and helminths (21.57%).

Al-Daoody[5] has collected 1258 stool samples from school children and foodhandlers in Ninevah governorate. The samples were examined by direct and concentration methods. The rate of infection among was 50.6% of which 29.8% were protozoa, 12.1% helminths and 8.7% mixed infections.

Abahussain [6] studied the prevalence of intestinal parasites among expatriate workers in Al-Khobar-Saudi Arabia, the results of 994 stool specimens showed that the rate of infection was 31.4%, of which 22.3% were single and 9.1% with multiple infections (double, triple and quadruple).

Çeliksöz et al. [7] carried out a study to determine the prevalence of parasites in three different regions (Alibaba, Esentepe, and Çayboyu) of Sivas, Turkey. Stool specimens were taken from 1864 participants during 641 household visits. The total prevalence was 37.2%.

The objectives of the present study are to know some information about the prevalence of intestinal parasites among food-handler workers and to compare several methods with respect to accuracy, diagnostic yield, time consumption and cost.

In Iraq, relatively, few studies have been done on intestinal parasites, and to the best of our knowledge no study has been done on the intestinal parasites among foodhandlers in Erbil city.

The objectives of the present study are:

1-To know some information about the prevalence of intestinal parasites among foodhandler workers in Erbil city.

2- To explore the various epidemiological factors obtained through different ways of fecal analysis.

METHODS

Sampling Area

During the period from the beginning of October 2005 to the end of March 2006, a total number of 587 stool samples were collected from male individuals representing different sectors of foodhandlers (460 Restaurant workers, 66 Refreshment workers, and 61 College club workers) of Erbil city. The fresh stool samples were taken immediately from the individuals then examined by both direct and concentration methods. Those with negative results two other samples were taken from them at different times for further examination.

Data Collection

Data about age and residence were recorded for each person on a special form together with stool samples obtained through different ways of fecal analysis.

Laboratory Examination

The stool samples were examined macroscopically (with the naked eyes) for its characteristics for consistency (formed, soft, loose, or watery), color (brown, yellow, green,...), texture, presence of mucus and blood, and also for the presence of any adult helminthes and the segments of tape worms, then examined microscopically by direct slide preparations (wet mount and Lugol's iodine 5%) for the presence of intestinal parasites[8,9] as well as formalin-ether sedimentation and zinc-sulphate methods were used according to the [10].

A small quantity of the selected fresh stool was placed on two separated warm slide with an applicator stick, thoroughly emulsified in one or two drops of warm physiologic solution for the first slide and also one or two drops of iodine solution for

the second slide, and mounted with a cover glass for each slide[9]. Using low power objective lens (10x), suspected objects are examined using the high-dry objective lens (40x). Three smears for each specimen were prepared and examined microscopically by this method.

Since direct method cannot detect all parasites particularly if the infection is light, concentration techniques were used as floatation (zinc-sulfate) and sedimentation (formalin-ether) techniques. In this method a solution of zinc-sulfate with a specific gravity of 1.180 gm/cm3 which was prepared by dissolving 331gm of zinc-sulfate in 1000 ml distilled water and adjusting to exact specific gravity using a Baume'-Density-Hydrometer T.P. at 20oc. And the steps was started by preparing 1 part of stool sample to 10 part of distilled water, then it was strained through wet gauze and centrifuged for 2 min. at 1500-2000 rpm, after discarding the supernatant, this step was repeated twice, the last supernatant fluid was poured off completely and 1 or 2 ml of zinc-sulfate solution is added. The packed sediment was broken up, and enough zinc-sulfate solution was added to fill the tube to 3 or 4 mm below the rim. The tube was centrifuged for 2 minutes at 1500-2000 rpm. After that the tube was removed from the centrifuge and avoiding agitation. Several loopfuls of the surface film were removed by means of a wire loop (4 mm in diameter) on to the clean slide, one drop of iodine stain was added, and the preparation was agitated manually to ensure uniform staining. The preparation was mounted with a cover glass and ready for examination under the microscope (5).

The same previous procedure was performed for sedimentation technique, but istead of zinc-sulfate it was used a biphasic technique (formalin and ether). Formalin used for fixing and preserving the fecal specimen and ether used to decrease the specific gravity of small fecal particles (5, 6).

Statistical Analysis

The results were analyzed statistically using the computer program SPSS 11.5 for Windows, for evaluating the differences according to different parameters chi-square (α2) test was used, in which calculated α2 compared with tabulated α2 at both levels (0. 01 and 0. 05) of significant. The differences were considered to be statistically significant when p-value obtained was less than 0.05.

RESULTS AND DISCUSSION

Throughout the period of this study several species of intestinal parasites (Protozoa and Helminthes) were identified among foodhandler workers in Erbil city center. The results of the present study showed the occurrence of several intestinal parasites of public health importance among foodhandlers.

Many epidemiological factors are associated with prevalence of intestinal parasites among different communities; of these factors are age, occupation, geographical distribution, sex and methods of diagnosis, which are taken into consideration in this study.

(Table 1) represents the male foodhandlers which were classified according to the rate of infection with different intestinal parasites.

Out of 587 male foodhandlers, 156 positive cases (26.58%) were reported in our study to harbor one or more intestinal parasites. As shown in (Table 2) out of the 460 restaurant workers, 110 cases (23.91%) gave positive results, among 60 refreshment workers showed 19 positive cases (28.79%) and for the 61 college club workers, 27 positive cases were reported and gave the highest rate of infection (44.26%).

The highest rate of infection was 12.95% (75 positive cases) for *E. histolytica*, the next species was *G. lamblia* with 47 positive cases (8.01%), followed by 19

cases (3.24%) of *E. coli*, only one positive case (0.17%) for both *I. buetschlii* and *T.*

Table 1. Rates of infection with intestinal parasites among foodhandlers: examination of 587 fecal samples in Erbil-province.

Parasites	Number of Positive samples	% from total Positive samples	% from total Number samples
Entamoeba histolytica	**75**	**48.08**	**12.95**
Entamoeba coli	**19**	**12.17**	**3.24**
Giardia lamblia	**47**	**30.13**	**8.006**
Iodamoeba buetschlii	**1**	**0.64**	**0.17**
Trichomonas hominis	**1**	**0.64**	**0.17**
Total protozoa	**143**	**91.66**	**24.53**
Hymenolepis nana	**12**	**7.69**	**2.04**
Ascaris lumbricoides	**1**	**0.64**	**0.17**
Total Helminths	**13**	**8.33**	**2.21**
Total Parasites	**156**	**100**	**26.58**

Table 2. Rates of infection from positive samples of each occupation with different intestinal parasites of foodhandlers in Erbil-province.

Parasites	Restaurant workers (460)		Refreshment workers (66)		College club workers (61)		Total (587)	
	No.	%	No.	%	No.	%	No.	%
Entamoeba histolytica	**54**	**11.74**	**9**	**13.64**	**12**	**19.67**	**75**	**12.77**
Entamoeba coli	**14**	**3.04**	**3**	**4.55**	**2**	**3.28**	**19**	**3.24**
Giardia lamblia	**32**	**6.96**	**5**	**7.58**	**10**	**16.39**	**47**	**8.01**
Iodamoeba buetschlii	**1**	**0.22**	**0**	**0**	**0**	**0**	**1**	**0.17**
Trichomonas hominis	**1**	**0.22**	**0**	**0**	**0**	**0**	**1**	**0.17**
Total protozoa	**102**	**22.17**	**17**	**25.76**	**24**	**39.34**	**143**	**24.36**
Hymenolepis nana	**7**	**1.52**	**2**	**3.03**	**3**	**4.92**	**12**	**2.04**
Ascaris lumbricoides	**1**	**0.22**	**0**	**0**	**0**	**0**	**1**	**0.17**
Total Helminthes	**8**	**1.74**	**2**	**3.03**	**3**	**4,92**	**13**	**2.21**
Total Parasites	**110**	**23.91**	**19**	**28.79**	**27**	**44.26**	**156**	**26.57**

(α2 = 26.38694)

hominis were reported. The total positive numbers of protozoa were 143 (24.53%), while for helminthes were 143 (2.21%) which included only one positive case (0.17%) of *A. lumbricoides* and 12 positive cases (2.04%) of *H. nana.*

Al-Kachache [4] in Mosul, Molan and Farag [3] in Erbil, Hussein [10] in the same city, Al-Daoody [5] in Mosul, Salih[11] , Abdullah et al. [12] , Farag[13] in Erbil and Al-Saeed et al. [14] in Dohuk, all recorded higher rates of infection than ours.

The above higher rates of infection compared to what is reported in the present study (26.8%), may be due to low socioeconomic conditions, personal hygiene, immune status, unsafe water supplies and abundance of some insects, which may act as carrier for these parasites, as part of these studies were done in rural regions.

Al-Shirifi [15] in Kirkuk recorded close rate to ours among foodhandlers, this could be due to the somewhat similar climatic factors, while the lower rates of infection compared to the present study were done by Jassim et al. [16] in Kirkuk, Kadir et al. [17] in Erbil, Al-Barzanjey[18] in Erbil, Salih[11]in Erbil and Hussein [19] in Sulaimani as the reasons explained by the authors, Kadir et al. [17] ascribe this to the high standard of living and better sanitation of the studied groups, while Hussein [19] believed that the low degree of temperature in Sulaimani district played a role in distribution of the parasites.

Many pathogenic intestinal parasites are transmitted through the faecal-oral route. Since unhygienic preparation, storage and handling of food by infected individuals are a major cause for food-borne diseases; foodhandlers need to be screened before they are allowed to work in food establishments such as restaurants, hotels, food stores, factories or as helpers and cooks in private houses[20].

Seven species of intestinal parasites were identified, in which *E. histolytica* showed the highest rate of infection among these parasites with the rate 48.08% from the total positive cases and 12.95% from the total number of samples, the latter is close to what has been reported by Hussein[(19)] in Sulaimani, while higher rates of infection with *E. histolytica* than our rate of infection were recorded by Koyee [21] in Erbil and Al-Saeed et al. [14] in Dohuk, Infection is transmitted via contaminated food and water, foodhandlers may carry amoebic cysts, therefore they play a role in spreading the infection, direct contact with infected feces also may be responsible for person-to-person transmission[22] .

Various rates of infection with this parasite were recorded in Iraq and other countries of the world in previous studies. Lower rates of infection were recorded by Shihab and Sultan [23] in Baghdad, Jassim et al. [16] in Kirkuk, Kadir et al. [17] in Erbil, Al-Barzanjey [18] in Erbil, Al-Daoody [5] in Mosul, Farag[15] in Erbil, Al-Shirifi [15] in Kirkuk, Hussein [19] in Sulaimani.

The differences could be due to what is mentioned by the authors, where they believed that health education, biological environment, poor hygiene status and abundance of flies can play a role in transmission of infection with *E. histolytica,* other factors may be due to the studied groups, in the present study, we examined asymptomatic foodhandlers, while most of the above high rates with this parasite due to the examination of hospitalized patients with symptoms.

The infection rate with *G. lamblia* was 30.13% among total positive of samples and 8.006% among total number of the samples. Close rate of infection with this parasite was recorded by and Hussein[19] in Sulaimani, lower rates than ours were recorded by Shihab and Sultan[23] in Baghdad, Jassim et al. [16] in Kirkuk, Al-Daoody [5] in Mosul and Farag[13] in Erbil.

The higher rates of infection than ours were recorded by Kadir et al. [17] in Erbil, Al-Kachache [4] in Mosul, Molan and Farag [3] in Erbil, Hussein [10] in the same city, Al-Daoody [5] in Mosul, Salih[11] , Abdullah et al. [12] , Farag[13] in Erbil and Al-Saeed et al. [14] in Dohuk, These higher rates of infection than the rate of the present study, could be due to the direct transmission of the parasite through contaminated food and water, or from person to person and/or the presence of some non-specific strains of *G. lamblia* (which have wide range of hosts) and the man will be sensitive to be affected by these strains that may be present in mammals or birds which act as reservoir hosts.

For non-pathogenic protozoa of the present study, the rate of infection with *E. coli* was recorded 12.17% among total positive cases and 3.24% among total number of the samples. The higher rates of infection with *E. coli* than the present study (3.24%) were recorded by Molan and Farag [3] in Erbil, Hussein[10] in the same city, Al-Daoody[5] in Mosul, Al-Shirifi [15] in Kirkuk and Farag[13] in Erbil. The relative high

rates of infection with this species reflect to the socio-economic status, availability of insects, water contamination, environmental conditions, or may be due to the rare distribution of this parasite in our region of the study and sanitation conditions of the studied areas.

Relatively close rate of infection with *E. coli* were recorded by Hussein [19] in Sulaimani, while the lower rates of infection compared to our rates of infection was recorded by Abahussain [6] in Saudi Arabia.

In the present study, it was recorded 0.17% of infection with *I. buetschlii*, the higher rates of infection with this parasite recorded by Al-Daoody [5] in Mosul, Al-Shirifi [15] in Kirkuk and Farag [13] in Erbil.

We live in a microbial world, and there are many opportunities for food to become contaminated as it is produced and prepared. Many foodborne microbes are present in healthy animals (usually in their intestines) raised for food. Meat and poultry carcasses can become contaminated during slaughter by contact with small amounts of intestinal contents. Similarly, fresh fruits and vegetables can be contaminated if they are washed or irrigated with water that is contaminated with animal manure or human sewage. In food processing, foodborne microbes can be introduced from infected humans who handle the food, or by cross contamination from some other raw agricultural product.

The rate of infection with *T. hominis* was 0.17%, higher rates of infection were recorded by Al-Kachache [14] in Mosul and Hussein [19] in Sulaimani. Beaver and Jung [24] showed that *T. hominis* has only a trophozoite stage; this fact may be responsible for the lower rates of infection.

The infection rate with *H. nana* was 2.04%, the relatively close rates of infection with this parasite were recorded by Al-Daoody [5] in Mosul, and Al-Saeed et al. [14] in Dohuk, while higher rates were recorded by Jassim et al. [16] in Kirkuk, Hussein[10] in Erbil, Farag [13] in the same city, and Al-Shirifi[15] in Kirkuk.

Lower rates of infection with this parasite were recorded by Shihab and Sultan [23] in Baghdad and Kadir et al. [17] in Erbil.

The rate of infection with *A. lumbicoides* was 0.17%, several studies were conducted in Iraq by several authors, and they have reported higher rates of infection with this parasite than the rate of infection in our study Hussein [10] in Erbil, Al-Daoody[5] in Mosul, Al-Shirifi [15] in Kirkuk, Al-Saeed [14] in Dohuk and Hussein[19] in Sulaimani.

The difference between the rates recorded by these authors in Iraq with the rate of infection in our study may be due to the low socioeconomic conditions, personal hygiene and the difference in environmental conditions. Similar rate recorded by Al-Barzanjey [18] in Erbil for this parasite.

As shown in the (Table 3), the infection was related with age groups; it is revealed that the infection rate was high in age group 10-19 years as it was 36.9% with different intestinal parasites, the lower rate recorded by Al-Kachache [4] in Mosul, Molan and Farag [3] in Erbil, Hussein [10] in the same city, Al-Daoody [5] in Mosul, Salih[11] , Abdullah et al. [12] , Farag[13] in Erbiland Al-Saeed et al. [14] in Dohuk and Hussein [19] in Sulaimani, and the differences between these rates of infection and our rate of infection is probably due to the methods of fecal analysis and sanitation level of the studied groups, *E. histolytica* showed

highest rate of infection (16.07%) among other parasites for this age group lower rate of infection were recorded by Al-Daoody [5] in Mosul, Al-Shirifi [15] in Kirkuk and Al-Saeed et al. [14] in Dohuk, the high rate of infection among peoples of this age group (10-19 years old) due to the high numbers of examined specimens and the age of the most workers situated in this age group. highest rate of infection (16.07%) among other parasites for this age group lower rate of infection were recorded by Al-Daoody [(15)] in Mosul, Al-Shirifi [15] in

Kirkuk and Al-Saeed et al. [14] in Dohuk, the high rate of infection among peoples of this age group (10-19 years old) due to the high numbers of examined specimens and the age of the most workers situated in this age group.

Table 3.Rates of infection with intestinal parasites among foodhandlers : examination of (587) fecal samples from total positive cases, examined in Erbil-province according to different age groups.

Parasites	10-19 Years (168)		20-29 Years (240)		30-39 Years (118)		40-49 Years (37)		50< Years (24)		Total (587)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
E. histolytica	27	16.07	31	12.92	10	8.47	6	16.22	1	4.17	75	12.78
E. coli	6	3.57	7	2.92	4	3.39	2	5.4	0	0	19	3.24
G. lamblia	20	11.9	22	9.17	3	2.54	2	5.4	0	0	47	8.01
I. buetschlii	0	0	1	0.42	0	0	0	0	0	0	1	0.17
T. hominis	1	0.59	0	0	0	0	0	0	0	0	1	0.17
Totalprotozoa	54	32.14	61	25.42	17	14.4	10	27.03	1	4.17	143	24.36
H. nana	8	4.76	4	1.67	0	0	0	0	0	0	12	2.04
A.lumbricoides	0	0	1	0.42	0	0	0	0	0	0	1	0.17
Total Helminths	8	4.76	5	2.08	0	0	0	0	0	0	13	2.21
Total Parasites	62	36.9	66	27.5	17	14.4	10	27.03	1	4.17	156	26.57

While the lowest rate of infection was 4.17% in age group over 50 years old, the highest total rate of both protozoan and helminthes infection among different age groups was recorded in the age group 10-19 years which was 32.14% and 4.76% respectively, followed by age group 20-29 years (2.08%), but there were no helminthes infection in other age groups (30-39, 40-49 and over 50 years old), among the protozoa, *E. histolytica* showed the highest rate (16.07%) of infection among the age group 10-19 years, while the lowest rate (4.17%) of this species was recorded among age groups over 50 years, the second high rate (11.9%) of protozoan infection was *G. lamblia* among age groups 10-19 years, whereas the low rate (2.54%) of this species was found among age groups 30-39 years, but there were no infection of this species over 50 years old, followed by *E. coli* the highest rate (5.4%) occurred in the age group 40-49 years and the lowest (2.92%) in the age groups 20-29 years, but there were no infection of this species over 50 years old and only one positive case (0.59%) reported of *T. hominis* among age groups 10-19 years old.

For the intestinal helminthes, only one positive case (0.42%) of intestinal nematode (*A. lumbricoides*) was reported and that was in the age group 20-29 years, while for the intestinal cestodee were 12 positive cases (2.04%) of *H. nana*, among this the highest 4.76% rate (8 positive cases) were reported in the age group 10-19 years and 4 positive cases (1.67%) among 20-29 years, but there were no infection in other age groups.

About the age groups of 20-29 years old, the rate of infection with different intestinal parasites among the total number of this age group was 27.5%, the lower rates of infection than the rate of the present study were recorded by Al-Kachache[4] in Mosul, Al-Saeed et al. [14] in Dohuk and Hussein [19]in Sulaimani, while Salih[25] in Erbil was recorded higher rates of infection than ours, this differences could be due to the climatic factors, personal hygiene and socioeconomic status. The predominant parasite in this age group was *E. histolytica* (12.92%) among total number of the samples; this could be due to the direct transmission of this parasite through contaminated fruits, vegetables and water.

The rate of infection from the total number of the samples for the age group 30-39

years old among foodhandlers was 14.4%, the lower rates of infection were recorded by Salih[25] in Erbil and Al-Saeed et al. [14] in Dohuk, close rate reported by Al-Kachache [4] in Mosul, while higher rate were recorded by Hussein [19] in Sulaimani and Salih [11] in Erbil, these differences of the rate of infection could be due to the difference in the environmental conditions and socioeconomic status.

E. histolytica showed the highest rate of infection (8.47%) among the other parasites in this age group, the lower rate of infection with this parasite recorded by Al-Saeed et al. [14] in Dohuk and Hussein[19] in Sulaimani.

The rate of infection with different intestinal parasites for the age group 40-49 years old was 27.03% and for the age group 50 years and above was 4.17%, when these two age groups are combined, the rate of infection was became 15.6% and this rate was lower than what was recorded by Salih[25] in Erbil and Al-Saeed et al. [14] in Dohuk, *E. histolytica* was the most predominant parasite (16.22%) for the age group 40-42 years and 4.17% for the age group 50 years and above, when these two rate of infection with *E. histolytica* are combined, the rate of infection was 10.2%, close rate of infection with this parasite to our rate was recorded by Hussein [19] in Sulaimani, while the lower rate recorded by Al-Saeed et al.[14] in Dohuk.

From (Table (4) showed number of infection according to the geographical distribution of foodhandlers, the highest total number of infection was (30 positive cases) recorded among those workers that located at the Shekhalla Street, this may be due to the daily mixing of population in this street and neglecting monthly stool examination of foodhandler workers, followed by both Kirkuk and Bata streets (26 positive cases), then both Tayrawa and Shorish streets (14 positive cases), the total positive numbers in Iskan, Tajel and Qala streets were 10 positive cases, while for 60 metry street 11 positive cases were recorded, but the lowest positive number only 5 cases were reported at the Setaqan street.

Table (4). Numbers of positive cases of different intestinal parasites among foodhandlers according to the geographical distribution of working in Erbil-city.

Parasites	Iskan Street	Setaqan Street	60 m Street	Kirkuk Street	Shekhalla Street	Tayrawa Street	Bata Street	Shorsh Street	Taiel Street	Qala Street	Total
E. histolytica	5	2	5	13	13	2	14	9	4	8	75
E. coli	1	1	0	2	9	1	5	0	0	0	19
G. lamblia	3	2	5	9	5	7	5	4	5	2	47
I. buetschlii	0	0	0	0	0	1	0	0	0	0	1
T. hominis	0	0	0	0	1	0	0	0	0	0	1
Total protozoa	9	5	10	24	28	11	24	13	9	10	143
H. nana	1	0	1	2	1	3	2	1	1	0	12
A. lumbricoides	0	0	0	0	1	0	0	0	0	0	1
Total Helminths	1	0	1	2	2	3	2	1	1	0	13
Total Parasites	10	5	11	26	30	14	26	14	10	10	156

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پوختە

پەتازانی مشەخۆرەکانی ڕیخۆڵە لەنێوان کرێکارەکانی خۆراك لە پارێزگای هەولێر

پێشەکی و ئامانجەکان: لێکۆڵینەوەیەکی پەتازانی مەیدانی ئەنجامدرا لە ماوەی نێوان سەرەتای مانگی تشرینی یەکەمی ساڵی 2005 تا کۆتایی مانگی ئازاری ساڵی 2006 بۆ زانینی جۆرو ڕێژەی بلاوبوونەوەی مشەخۆرەکانی ڕیخۆڵە لە نێو کرێکارەکانی خۆراك لە پارێزگای هەولێر.

ڕێگاکان: کۆی گشتی 587 ژمارەی نمونەی پیسایی لە کرێکارەکانی خۆراك کۆکراوەکان کە تەمەنیان لە نێوان 10–50 ساڵ وە زیاتر لە 50 ساڵ دا بوون. بۆ ئەم مەبەستە نمونەکان شیکارکران بەهەردوو ڕێگای ڕاستەوخۆو ناڕاستەوخۆ (خەستی)، ئەمەی دوایی ڕێگەی سەرئاوکەوتن لە گۆگرداتی زینگ، گیراوەی شەکر وە گیراوەی خوێ ی خەست لەگەڵ ڕێگەی نیشتن لە فۆرمالین–ئیسەر دەگرێتەوە.

ئەنجام و دەرئەنجامەکان: ڕێژەی سەدی گشتی لەنێوان کرێکارەکانی خۆراك (26.5٪) بوو، کە (24.53٪) ی تووش بوون بە سەرەتاییەکان Protozoans و (2.21٪) ی تووش بوون بە کرمەکان Helminthes بوو. ڕێگەی سەرئاوکەوتن بە گۆگرداتی زینگ بەرزترین چوستی پیشاندا لە دەستنیشان کردنی مشەخۆرەکانی ڕیخۆڵە، بەدوایدا ڕێگەی سەرئاوکەوتن بە گیراوەی خەستی خوێ ی چێشت بە پلەی دووەم دێت، جیاوازی لە نێوان ئەو ڕێگایانە لەڕووی ئاماریەوە واتایی بوو لە هەردوو ئاستی 0.01 وە 0.05 . لەم ڕووپێوەدا کە لە نێوان کرێکارەکانی خۆراك ئەنجام درا ئەم مشەخۆرانەی خوارەوە دەرکەوتن: لە نێو سەرەتاییەکاندا ئەمیبای زەحیری *Entamoeba histolytica* بەرزیرین ڕێژەی (12.95٪) بلاوبونەوەی هەبوو، بەدوایدا جیاردیا لامبلیا *Giardia lamblia* کە (8.006٪) بوو، ئەمیبای کۆلۆن *Entamoeba coli* کە (3.24٪) بوو وەکەمترین ڕێژەی توشبوون (0.17٪) بوو بۆ هەردوو جۆر لە ئایۆدامیبا بۆشلای *Iodamoeba buetschlii* وە ترایکۆمۆناس هۆمینس *Trichomonas hominis*. لەنێوان تووش بوون بە کرمەکان کرمی هایمینۆلیپس نانە *Hymenolepis nana* بەرزترین ڕێژەی (2.04٪) توشبوونی هەبوو وە نزمترین ڕێژەش (0.17٪) بۆ کرمی ئەسکارس لامبریکۆیدس *Ascaris lumbricoides* تۆمارکرا. بەرزترین ڕێژەی توش بوون (21.29٪) بە یەك جۆر مشەخۆربوو، ڕێژەی توشبوونی دوانی (2.55٪) بوو، وە تەنها لە یەك باردا توشبوونی سیانی بە ڕێژەی (0.17٪) لە کۆی توشبوان تۆمارکرا. جیاوازی واتایی تێبینی نەکرا بۆ توشبوون لە کۆمەڵە جیاوازەکانی تەمەن لە نێوان کرێکارەکانی خۆراك" هەرچەندە جیاوزیەکی ڕێژەیی کەم تێبینی کرا بەڵام واتایی نەبوو لەڕووی ئاماڕەوە.

الخلاصة

وبائيّة الطفيليّات المعويّة بين العاملين في مجال الأغذية في محافظة أربيل

الخلفية والاهداف: اجريت دراسة وبائية خلال الفترة من بداية شهر تشرين الأول 2005 وحتى نهاية شهر اذار 2006 لتقصي نمط وإنتشار الطفيليات المعوية بين العاملين في مجال الأغذية في محافظة اربيل.

الطرق : تم جمع 587 عينة براز من العاملين في مجال الأغذية الذين تتراوح اعمارهم بين 10–50 سنة واكثر من 50 سنة . تم فحص عينات البراز بالطريقة المباشرة وطرق التركيز (التعويم والترسيب) . تم استخدام كبريتات الزنك, السكر, والمحاليل الملحية في التعويم بينما استخدم الفورمالين-ايثر في الترسيب.

النتائج والاستنتاجات : بلغت نسبة الاصابة الكلية بين العاملين في مجال الأغذية (26.58٪)، من بينها (24.5٪) اصابات بالأوالي Protozoans و (2.21٪) اصابات بالديدان الطفيلية Helminthes .

اظهرت طريقة التعويم بواسطة كبريتات الزنك كفاءة عالية في تشخيص الطفيليات المعوية, تليها طريقة التعويم وباستخدام المحلول الملحي المشبع, وكانت الفروقات واضحة احصائيا بين الطريقتين وبمستوى 0.01 و 0.05 .

أظهرت المسحات التي اجريت بين العاملين في مجال الغذاء وجود الانواع التالية من الطفيليات :من بين الأوالي Protozoans كانت النسبة العالية للأميبا الحالّة للنسيج *Entamoeba histolytica* (12.95٪) وتليها جياردیا لامبليا *Giardia lamblia* بنسبة (8.006٪) , اميبا القولون *l Entamoeba coli* (3.24٪) وكانت نسبة الاصابة اقل بايوداميبا بوشلاي Iodamoeba *buetschlii* والمُشعرة البشرية *Trichomonas hominis* (0.17٪).

من بين الاصابات بالديدان الطفيلية كانت النسبة الاكبر للمُحرشفة القزمة *Hymenolepis nana* التي بلغت (2.04٪) واقل نسبة كانت لأسكارس لمبريكويدس *Ascaris lambricoides* (0.17٪).

كانت نسبة الاصابة الطفيلية الاحادية هي الاعلى والتي بلغت (21.29٪) وتليها الاصابات الطفيلية المزدوجة بنسبة (2.55٪) وتم تسجيل حالة اصابة طفيلية ثلاثية واحدة بنسبة (0.17٪) من مجموع الاصابات الكلية التي سجلت.

لم يتم ملاحظة فروقات واضحة باختلاف الفئات العمرية بالرغم من وجود بعض الفروقات لكنها لم تكن ذات اهمية احصائية.

TINIDAZOL SOLUTION AS A NEW SCOLICIDAL AGENT IN HYDATID CYST SURGERY

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ABSTRACT

Objectives: To evaluate the effect of Tinidazol solution as a direct injectable scolicidal agent inside the hydatid cyst.
Design: prospective
Settings: Mosul University, Mosul, Iraq. Emergency Teaching Hospital, Duhok, Iraq
Subjects: Twenty volunteer patients with hydatid disease of the liver, spleen and pancreas were included in this study conducted between June 2003 and June 2011.
Intervention: The prepared Tinidazol solution was used as an intracystically injectable scolicidal agent peroperatively in quantities corresponding to the cysts' sizes for ten minutes. The scolices viability was established by checking the movement and activity of flame cells, disrupted membrane or stained with eosin is considered as dead.
Results: Postoperatively all subjects have good recovery, no complications, with normal serological diagnostic test effects, no sign and symptoms of cholangitis, hepatitis or recurrence within six months.
Main outcome measure: The microscopical examination by the immediate eosin staining after the Tinidazole injection revealed non-viable protoscolices. The histopathological examination of serial sections of the cysts wall after the cysts removal, revealed laminated germinal layer of hydatid cyst and no scolices at all.
Conclusion: Tinidazol solution after its preparation can be used as a directly injectable scolicidal agent for sterilization before its removal with no evidence of any complications.

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Key words: hydatid disease, scolices, scolicidal agents, Tinidazole

Hydatid Disease is a parasitic disease that affects both humans and other mammals by the larval stages of the cestode (tapeworm) species, is called echinococcosis (hydatid cyst) [1]. There are three different forms of echinococcosis found in human, each of which is caused by the larval stages of different species of the tapeworm of genus *Echinococcus* [2,3]. In Iraq mainly *Echinococcus granulosus* had been found [4]. The wall of the parasitic cyst has two structural components; an outer acellular laminated membrane and an inner germinal membrane where the protoscolices bud from it [5]. In addition, the human affected organ will form an outer adventitial layer wrapping the parasitic cyst.

The hydatid disease is an endemic disease in the Mediterranean countries and the Middle East especially in Iraq [4].

Surgery remains the primary treatment and the only hope for complete cure for hydatid cyst of the liver since the results of medical and percutaneous treatment are still controversial [3]. In the surgical management of this disease, sterilization of the cyst cavity by injection of a scolicidal agent, protection of the surrounding tissues, evacuation of the cyst and the management of the residual cavity are the main principal steps [6,7].

Many Scolicidal agents include formalin, hydrogen peroxide, hypertonic saline, chlorhexidine, absolute alcohol, and cetrimide have been used and a variety of complications have been described with all these scolicidal agents [8].

Tinidazol solution has not been used before as a directly injectable scolicidal

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agent inside the hydatid cyst peroperatively for sterilization before its removal.

This study aims to evaluate in an in-vivo basis the effectiveness of Tinidazol solution as a directly injectable scolicidal agent inside the liver hydatid cyst intra-operatively for sterilization before its removal which was not used before intra-cystically.

METHODS

This prospective study was conducted between June 2003 and June 2011 and carried out on twenty volunteer patients with signed agreement certification, illustration of the procedure and explanation of the possible complications suffering from operable hydatid disease of the liver, spleen and pancreas and did not received any medical or surgical treatment for the hydatid disease before confirmed by ultrasonic and computerized tomographic studies, of different ages, sex, cyst number and sizes as shown in (Table 1) in The Emergency Teaching Hospital (Duhok city, Kurdistan region – Iraq).

The Tinidazole solution prepared by dissolving 5 grams of Tinidazole hydrochloride in water for injection up to 1000 ml then filtered and filled in amber container with 100ml for each container, closed with stopper and sterilized with autoclave at 116˚ C, pressure at 0.75 Bar for 40 minutes as in the standard preparation and concentration of the intravenous Tinidazole vial solution (500 mg/100 ml) which is not available in Iraq.

A spectrophotometric method was used to determine the concentration at wavelength 311nm. pH measures (5.5-7). The solution was tested on Albino rabbit and showed negative results for increasing in the body temperature means neither pyrogens nor microbes were present.

The method of shelf- life predication based on 3 months accelerated stability data was utilized. The degradation of the Tinidazole hydrochloride behaves accordingly to first order kinetics. The time for the loss lines at 40 ˚C & 60 ˚C to reach 90% of the initial concentration is labeled. The solution is stable at least 2 years. Protected from light, at a temperature below 30 ˚C[9,10].

This solution was tested in vitro on ten intact viable hydatid cysts of sheep for different periods of time to determine the time needed for Tinidazole to inactivate the sheep`s hydatid cysts, after histological examination by 0.1% eosin dye[11-13] it had been found that ten minutes is the least effective time to kill all the scolices.

The prepared Tinidazol solution was used as an intracystically injectable scolicidal agent peroperatively for inactivation of the fertile hydatid cyst before its removal in quantities corresponding to their sizes and maximum intracystic tension reached without leakage which differ from case to another accordingly for ten minutes, as shown in (Table 2).

During surgery, the exact location of the cyst was identified. The surrounding tissues were protected by covering them with Tinidazol solution-soaked pads. The cystic fluid was sampled by syringing (sample No. 1) and then partially evacuated using a suction device, and Tinidazol solution was instilled to its maximum tension without leakage in the cavity and allowed to sit for 10 minutes, then after was sampled again (sample No. 2) and then completely evacuated. Then the outer adventitial layer was opened and the parasitic cyst and its content were removed and sampled (sample No. 3). Care was taken to ensure no spillage occurs to prevent seeding and secondary infestation.

At time of operation, after preparation of the three samples, they were transferred to a microscope slide to which a drop of 0.1% eosin dye [11] was added. Viability was established by checking the movement and activity of flame cells, disrupted membrane or stained with eosin is considered dead [12,13].

RESULTS

In this prospective study twenty cases of hydatid disease of the liver and the spleen in the human were diagnosed, managed and finished follow up for six months between June 2003 and June 2011 in Duhok Emergency Teaching Hospital and the samples were studied in association with the parasitological consultation team.

The ages ranged from (10) years to (45) years with mean age (30.75) and the male to female ratio was 1:3 as shown in (Table 1).

Regarding the sites of the hydatid cyst were 16 patients in the liver (80%), 2 patients in the spleen (10%), 1 patient in the pancreas (5%) and 1 patient in both the liver and the spleen (5%). The number of the cysts varied from single cyst as in 14 patients (70%) to multiple cysts as in 6 patients (30%) with variable sizes ranging from 3x3 to 15x14. (Table 1).

Table 1. The patients; ages, sex and cysts, sites

Case No.	Age	Sex	Cyst site	Cyst No.	Cyst size
1	10	female	Liver	2	13x9 , 5x5 cm
2	40	male	Liver	1	10x10 cm
3	20	female	Pancreas	1	7x5 cm
4	38	female	Liver, spleen	2	10x9 , 8x7 cm
5	25	female	Liver	1	9x7 cm
6	27	male	Liver	4	7x5 ,5x5 , 3x4 , 3x3 cm
7	32	female	Liver	1	7x7 cm
8*	42	female	Liver	1	9x8 cm
9	40	female	Liver	1	15x14 cm
10	30	male	Spleen	1	15x10 cm
11	45	female	Liver	1	5x4 cm
12	34	female	Liver	3	10x8 , 8x7 , 6x6 cm
13	30	female	Liver	1	15x9 cm
14	24	female	Liver	1	9x8 cm
15	29	female	Spleen	1	13x10 cm
16	37	male	Liver	4	6x5 , 5x5, 5x5 , 3x4 cm
17	41	female	Liver	2	14x12 , 9x8 cm
18	35	male	Liver	1	15x11 cm
19	15	female	Liver	1	11x9 cm
20	21	female	Liver	1	12x10 cm

*** bile stained hydatid cyst fluid**

Regarding the fluid of the cysts; all cysts showed clear fluid except single case (case no.8) showed clear bile stained fluid.

The microscopical examination by the immediate eosin staining before Tinidazole injection of all (samples no. 1) revealed viable protoscolices. While the microscopic examination of (samples no.2) after the Tinidazole injection revealed non-viable protoscolices. (Table 2).

The histopathological examination of serial sections of the cysts wall after the cysts removal which referred as (sample no. 3); all revealed laminated germinal layer of hydatid cyst and no scolices at all. (Table 2).

DISCUSSION

The hydatid disease is a worldwide important medical issue with high endemicity in Iraq [4]. The surgical removal remains the main line of treatment and the scolicidal agents that used in the hydatid cyst operation are a major surgical problem. Many scolicidal agents have been used with variable degrees of success and complication [7] intraoperativily and postoperatively. Among the various most commonly known scolicidal agents are formalin which cause toxicity and no longer used [14],

Table 2. The injected Tinidazole quantity, waiting duration and the scolices viability.

Case No.	Cyst No.	The injected Tinidazol quantity (in ml.)	Waiting duration after injection (in minutes)	scolices viability after injection
1	2	10 , 3	All 10	All Dead
2	1	15	10	Dead
3	1	10	10	Dead
4	2	20 ,10	All 10	All Dead
5	1	10	10	Dead
6	4	7, 7, 5, 5	All 10	All Dead
7	1	10	10	Dead
8*	1	15	10	Dead
9	1	20	10	Dead
10	1	20	10	Dead
11	1	7	10	Dead
12	3	20, 15, 15	All 10	All Dead
13	1	25	10	Dead
14	1	15	10	Dead
15	1	25	10	Dead
16	4	10, 10, 10, 7	All 10	All Dead
17	2	25, 15	All 10	All Dead
18	1	25	10	Dead
19	1	20	10	Dead
20	1	25	10	Dead

** bile stained hydatid cyst fluid*

ethyl alcohol that is usually preferred for ultrasonic-guided percutaneous aspiration, injection and reaspiration (PAIR) of hydatid cysts [15,16] which cause sclerosing cholangitis [17], polyvinylpirrolidone (Betadine®) which cause renal shutdown, sterile peritonitis and sclerosing cholangitis [18], cetrimide which cause sclerosing peritonitis [19], metabolic acidosis [20] and methemoglobinemia[21], hydrogen peroxide and hypertonic saline which both have low efficacy and various complications [22,23].

As Mebendazole is no longer recommended for the treatment of hydatid disease by the FDA due to its neutropenic side effect [24]and Albendazole is already studied as scolicidal agent and shown not to be superior to other scolicidal agents [25]. Tinidazole which is also imidazole derivative and produces antiprotozoal effect was prepared as Tinidazole hydrochloride 500mg / 100ml Solution. This solution had been made approximately isotonic solution with pH ranging (5.5-7) [9,10] .A trial of injecting Tinidazole solution into the hydatid cyst and preserving the operative field with sponges soaked with this solution have been made to avoid dissemination of parasites during the surgery in all the twenty patients. The microscopical examination of the cystic fluid by the immediate eosin staining[11-13]revealed non-viable protoscolices and the histopathological examination of serial sections of the cysts wall after the cysts removal revealed laminated germinal layer of hydatid cyst and dead scolices at all cases. Postoperatively have good recovery, no fever or cardiovascular and neurological complications, with normal serological diagnostic test, no sign and symptoms of cholangitis or hepatitis in all the cases including (case no.8 which had bile stained fluid indicate possible connection with biliary system).

Follow up of the patients after their surgery by the ultrasound study and the liver function tests (serum total bilirubin, GPT and GOT) every six months and one computerized tomography scan at the end of the first six months reveled no

dissemination or recurrence of the hydatid disease with normal serological tests until June 2011.

Tinidazole tablets rapidly and completely absorbed, protein binding; 12%. Distributed in all body tissues and fluids; cross blood-brain barrier; significantly metabolized; Excreted primarily in urine and partially eliminated in feces. Half-life: 12-14 h with minimal and non serious side effects [26].

The intravenous infusion of Tinidazole, a mean 63% of the dose will be excreted in the urine, 12% of the dose will be excreted in the faces, indicating the possible involvement of biliary excretion[27]without any reports of cholangitis.

CONCLUSION

Although of extensive use of different scolicidal agents, a variety of complications have been described with all these scolicidal agents. We present evidences that Tinidazol solution after its preparation can be used as a safe directly injectable scolicidal agent inside the hydatid cyst peroperatively for sterilization before its removal with no evidence of any complications.

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پوختە

بکارئیناناگێراوێ تینیدازول وەک کوژەرێ کرمێن کیسێن ئاڤێ بڕێکا نشتەرگەریێ

ئارمانج: شیانێن بکارئینانا گێراوێ تینیدازول پشتی دروستکرنا وی وەک گێراوەکێ بکێرهاتی بو دەرزیك دانانا ئێکسەر و وەک کوژەرێ کرما د ناف کیسکێن ئاڤێ دا.

دیزاین: خاندنا چاڤەرێکری.

جهـ : زانکویا میسل ل باژێرێ میسل و نەخوشخانا تەنگاڤیا یا فێرکرنێ ل باژێرێ دهوکی.

بابەت:– 20 نەخوشێن خوبەخش ب نەخوشیا کیسکێن ئاڤێ ییێن مێلاکا رەش ڤێ خاندنێ ب خوڤە گرتن کو هاتبو ئەنجامدان دنافبەرا خزیرانا 2003 – خزیرانا 2011.

دەست تێوەردان: گێراوێ تینیدازول ییێ دروستکری وەک گێراوەکێ ئێکسەر, بکێربهێت بو دەرزیك دانانێ , و کوژەرێ کرمان هاتە بکارئینان د ناف کیسێن ئاڤێ دا ل دەمێ نشتەرگەریێ ژبو تعقیمکرنا کیسێ رخاو (خصب) بەری لادانا وی ب برەکا گونجای دگەل قەبارێن وێ بو ماوێ (10) دەقیقان, و چالاکیا کرمان بدەستڤە هات بڕێکا تاقیکرنا لڤاندن و چالاکییا خانێن لەهەبی, تێکچوون و رەنگدانا پەردێ ب رەنگێ ئیوزین دبیتە نیشانا مرنا وێ.

ئەنجام:– پشتی ئەنجامدانا کریارێن نشتەرگەریێ هەمی نەخوش د بارێن باش دا بوون, چ ئالوزی نەبوون, و هەمی تاقیکرنێن وان ییێن (سریری) د سروشتی بوون و چ سیمایێن ئیلتیهابا کەنالێن صەفراوی (القنوات الصفراویه) یان ئیلتیهابا مێلاکێ یان زڤرینا نەخوشیا کیسێن ئاڤی دیار نەبوون.

کاریگەریا ئەنجامی یا سەرەکی:– تاقیکرنا میکروسکوپی یا بلەز ب رێکا بویاغێ ئیزوین پشتی دانانا تینیدازولی دیارکر کو کرم نە د ساخن, و تاقیکرنا نەسیجی یا نەخوشیێ ژبو زنجیرا پارچێن دیوارێ کیسکی پشتی راکرنا وی دیارکر کو تەخێن جەرسومی ییێن (مصفح) ژ کیسێن ئاڤێ دیار بوون بەڵێ بێ هەبوونا کرمێن ساخ.

دەرئەنجام:– بکارئینانا ماددێ تینیدازول پشتی دروستکرنا وی وەک گێراو و دەرزی لێدانا وی دناف کیسێن ئاڤێ دا بڕێکەکا راستەوخو وەک کوژەرەکێ کرمان د ماف کیسێ ئاڤێ دا ل دەمێ نشتەرگەریێ ژبو تعقیمکرنا وی بەری لابرنا وی, و چ نیشانێن ئالوزییێ دیار نەبوون.

الخلاصة

استعمال محلول التندازول كقاتل لديدان الأكياس المائية جراحيا

الأهداف: امكانية إستعمال محلول التينيدازول بعد تحضيره كمحلول قابل للحقن المباشر و قاتل للديدان داخل الاكياس المائية.

التصميم: الدراسة المتوقعة.

الاماكن: جامعة الموصل في مدينة الموصل, ومستشفى الطواري التعليمي في دهوك, العراق.

المواضيع: عشرون متطوّع مريضِ بمرض الأكياس المائية الكبدية تُضمّنَ في هذه الدراسةِ أجرتْ بين يونيو/حزيرانِ 2003 ويونيو/حزيرانِ 2011

التدخّل: .إستُعملَ محلول التينيدازول المُحضَر كمحلول مباشر قابل للحقن قاتل للديدان داخل الكيس المائي خلال العملية الجراحية لتعقيم الكيس الخصب قبل إزالته بكميات متناسبة مع أحجامه لمدة(١٠) دقائق. إستُحصلَت حيوية الديدان بفحص حركة و فعالية الخلايا اللهبية, تمزق الغشاء أو الإنصباغ بصبغة الإيوزين تعبر عن موتها.

النَتائِج: بعد إجراء العمليات الجراحية كان جميع المرضى في حالة تحسّنُ جيدُة، لا تعقيداتَ، وكانت جميع فحوصاتهم السريرية طبيعية ولم تظهر عليهم أعراض التهاب القنوات الصفراوية أو التهاب الكبد أو عودة مرض الأكياس المائية.

إجراء النتيجةِ الرئيسي: الفحص المجهري الفوري بصبغة الإيوزين بعد حقن التينيدازول كشف أن الديدان غير حية. الفحص النسيجي المرضي لسلسلة مقاطع من جدار الكيس بعد إزالته كشف عن طبقات جرثومية مصفحة من الكيس المائي بدون أي ديدان حية على الإطلاق.

الاستنتاج: استعمال مادة التينيدازول بعد تحضيره كمحلول وحقنه داخل الأكياس المائية مباشر كقاتل للديدان داخل الكيس المائي خلال العملية الجراحية لتعقيمه قبل إزالته, بدول أي دليل على تعقيدات.

EPIDEMIOLOGY OF FUNGAEMIA AND ASSOCIATED RISK FACTORS AMONG PATIENTS IN A TERTIARY CARE HOSPITAL IN SAUDI ARABIA

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ABSTRACT

Background and objectives This study was conducted in-order to assess the epidemiology of fungaemia among hospitalized patients and to identify the risk factors associated with fungaemia in critical care units.

Methods: Blood cultures (24087 bottles) from all medical units at high infective risk mainly from intensive care unit and Nursery wards in Ohud Hospital in Madina Al-Munawara in Saudi Arabia were sent to Microbiology laboratory. A minimum of 5 mL of blood was collected aseptically by skin venepuncture and inoculated into a BacTec aerobic vial (Becton Dickinson) and incubated for 7 days. All positive cultures were inoculated on Sabouraud glucose agar (SGA) and incubated at 30 0C. An aliquot was Gram-stained for preliminary identification of the microorganism. *C. albicans* were identified based on colonial morphology and germ tube formation.

Results: A total of 115 cases of blood culture positive for fungi were identified out of 2875 positive bottles during 7 year period (2005-2011). The majority of episodes were due to *Candida albicans*: 60 (52%) followed by *candida non-albicans*: 53 (46%), and only 2 cases (2%) of non- *candida spp (Aspergillus spp*). The overall mortality was 32%.

In Nursery ward there were 59 (51%) cases of yeast in blood culture, among these episodes, 48 (81%) were positive for *Candida albicans*, and 11 (19%) were *Candida non-albicans*. The two main risk factors were: first prematurity and Respiratory Distress Syndrome (RDS): 48%. Second the use of 2 antibiotics combined or more: 71%. In adult Intensive care unit and other medical wards there were 56 (49%) positive cases. 12 (21%) episodes were *Candida albicans*, 42 (75%) were *Candida non-albican*s and only 2 (4%) were non-*candida spp* (*Aspergellus spp*). The associated risk factors in ICU were: diabetes: 18 cases (39%), respiratory disorders: 9 cases (20%), CVA: 6 cases (13%), post –operative; CNS infection and liver disorders: 3 cases (6.5%) each. There were also 2 cases (4.5%) for each of anaemia and septic shock.

Conclusions: this study highlighted the importance of candidemia among hospitalized patients. There was high percentage 81% of *Candida albicans* among fungaemic cases in Nursery Unit but *Candida non-albicans* constituted 75% among fungaemic cases in adult ICU.

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Key words: Fungaemia,Tertiary care Hospital, Saudi Arabia.

Candidal bloodstream infections (CBSIs) currently represent the fourth most common nosocomial bloodstream infections [1].The incidence of candidemia has risen over the last two decades, in several parts of the world and indifferent settings[2,3] . Mostly is due to an increase in the use of more aggressive therapy practices in intensive care units.

Systemic *candida* infections are associated with a high mortality rate (38%) and a prolonged hospital stay [4,5] . Therefore, in patients with accumulating risk factors for candidemia, in whom the probability for candidemia is high, clinicians use empirical treatment long before laboratory results on species identification and antibiograms are available, when the infection is suspected[6] . In order to apply an effective treatment, it is essential to have a valid, regularly updated knowledge of the proportion of *Candida spp* causing candidemia.

A significant variability has been noticed within studies, especially in multicenter studies enrolling patients in different settings [7].

Certain patients are at high risk of developing candidemia especially those receiving broad-spectrum antibiotics,

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chemotherapy, or steroids. In addition, the presence of central venous catheters (CVC), administration of total parenteral nutrition (TPN), complicated abdominal surgery, malignancy, neutropenia, and acute renal failure (ARF) are additional risk factors[8,9] .

Historically, *Candida albicans* accounts for 50—70% of all episodes of candidemia[10]. The prevalence of *C. non-albicans* is increasing in many centers[11].

This study compared *C. albicans* and *C. non albicans* in relation to the predisposing factors in different wards in Ohud Hospital mainly in ICU and Nursery wards.

METHODS

Ohud hospital in Madina al-Munawara in Saudi Arabia is a tertiary care hospital with a capacity of 270 beds. It comprises all branches of medical care including ICU and nursing wards.

A minimum of 5 mL of blood was collected aseptically by skin venepuncture for adults and 1-3 ml for paediatric patients. The blood was inoculated into a BacTec aerobic vial (Becton Dickinson). Specimens were incubated at 35 0C with continuous agitation for seven days. Positive cultures were manually sampled and inoculated on Sabouraud glucose agar (SGA) and incubated at 30 0C. An aliquot was Gram-stained for preliminary identification of the microorganism. *C. albicans* , *C. non-albicans* and *non-candida spp* were identified based on gram staining, colonial morphology and germ tube formation. A germ tube is an outgrowth produced by spores of spore-releasing fungi during germination. The germ tube differentiates, grows, and develops by mitosis to create somatic hyphae. A germ tube test is a diagnostic test in which a sample of fungal spores are suspended in serum and examined by microscopy for the detection of any germ tubes. It is particularly indicated for colonies of white or cream color on fungal culture, where a positive germ tube test is strongly indicative of *Candida albicans*. Candidemia was defined as a positive blood culture for *Candida spp* and the presence of signs and symptoms of sepsis. For the identified cases, we collected the demographic data (age and ward), underlying disease, and the presence of risk factors (neutropenia, surgery, and prior use of antimicrobial agents) of patients.

RESULTS

The overall number of fungaemia between 2005 and 2011 was 115 (4% of all significant blood stream infections). *Candida albicans* was the most commonly isolated species detected in 60 cases (52%). *Candida non-albicans* was detected in 53 episodes (46%) and the number for *non-candida (Aspergellus spp*) was 2 (2%). All the 115 bottles did not grow bacteria.

There were 59 episodes (51%) from Nursery ward, 49 out of 59 (87%) were neonates. The Number of *candida albicans* was 48 (81%). The number of *candida non-albicans* was 11 (19%).

The associated risk factors in Nursing ward in relation to fungaemic cases are: 23 out of 48 positive cases (48%) were premature and Respiratory Distress Syndrome (RDS). 34 out of 48 (71%) of cases were on 2 or more than 2 antibiotics including Vancomycin and or Tienam. There are no prior data or study for comparison.

The number of episodes from ICU and related medical wards were 56 (49%). 83% of cases were more than 60 years of age. Candida albicans were seen in 12 cases (21%), *candida non-albicans* were detected from 42 bottles (75%). There were 2 cases of *non-candida* (2%).

The associated risk factors in ICU in relation to fungaemic cases are shown in (Table 1).

Table 1.The associated risk factors in ICU in relation to fungaemic cases .

Risk Factor	Number of cases	%
Diabetes mellitus	**18**	**39**
Respiratory disorders	**9**	**20**
CVA	**6**	**13**
Post-operative	**3**	**6.5**
Meningitis	**3**	**6.5**
Liver disorders	**3**	**6.5**
Anaemia	**2**	**4.5**
Septic shock	**2**	**4.5**
Total	**46**	**100**

DISCUSSION

In this study, we evaluated the epidemiology and risk factors for fungaemia at a general hospital in Saudi Arabia. There were a total of 115 distinct episodes of fungaemia in the study period from 2005-2011. The total number of episodes was low for the seven-year study period. Although the culture system employed (BACTEC) is an adequate system for the detection of Candida, it is not the highest yield system available. A second reason for low incidence might be the types of patients seen in Ohud hospital as it does not have patients with organ transplants. Moreover there were no prior data or study for comparison.

The prevalence of the different species of Candida in blood stream infections varies from one institute to another. The most frequent species causing fungemia in our study was *C. albicans* and constituted 52% of all the isolates. In other studies from Saudi Arabia, the predominance of *C. albicans* from blood isolates has also been 46% [12] and 50 %[13] . In a study from a teaching hospital in the Eastern province of Saudi Arabia, C. albicans fungemia constituted only 19% of the total isolates [(14)]. Some other studies described the predominance of *non-C. albicans*[15] .

Antibiotic use is a known predisposing factor for the development of fungemia [14] This important risk factor was not assessed in some studies from Saudi Arabia [13] . In this study, the predisposing factors for the development of candidemia in Nursing ward was first the use of 2 or more of antibiotics in 71% of patients including vancomycin, Tienam or 3rd generation Cephalosporines. Second, prematurity with or without RDS and the association was 48%.

Candidemia-associated mortality has been found to be 31—72% [13,16,17] Similarly, the crude mortality rate in our study was 32% for all patients with candidemia, 29% for *C. albicans* and 26% for *non-C. albicans.* In a study from Italy, the mortality rate due to candidemia was 14.2% and 40% in the *C. albicans* and *non-C. albicans* groups, respectively 18. In another study, mortality associated with *C. albicans* and *C. parapsilosis* was 39.5% and 11.1%, respectively [19]. The difference in the observed mortality rates between these studies may be related to the severity of the underlying diseases.

Intensive care unit stay with its associated invasive procedures constituted an important risk factor of candidemia [21] . Other risk factors include severity of illness which promotes yeast over-growth and the increase use of potent antibiotics will cause disturbance of normal gut flora 21. In our study there were 56 (49%) positive cases. 12 (21%) episodes were *Candida albicans*, 42 (75%) were *Candida non-albicans* and only 2 (4%) were *non-candida spp (Aspergellus spp).* The associated risk factors in ICU were: diabetes: 18 cases (39%), respiratory disorders: 9 cases (20%), CVA: 6 cases (13%), post –operative; CNS infection and liver disorders: 3 cases (6.5%) each. There were also 2 cases (4.5%) for each of anaemia and septic shock.

In conclusion, the study shows that Most of our candidemic episodes occur in high risk, critical care units (ICU and neonatal unit). *C. albicans* was the major species causing fungemia followed by *C. non-albicans.* The crude mortality rate was 32% and two independent associated risk factors were the overuse of antibiotics and the prematurity in Nursing unit. In ICU

unit there were risk factors associated with candidaemia especially among diabetic patients. The *candida non-albicans (candida spp)* were not further classified thus the lack of these data is one of the limitations of the current study. This study highlights the importance of candidemia among hospitalized patients in critical care units and further studies are needed to determine the change in the pattern of candidemia and to include susceptibility testing.

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پوخته

به لاڤ بونا كه دووا دناڤ خينێ د ا وێه و هوكارێن مه ترسى دار دگه ل دا لده ڤ نه خوشێن نه خوشخانه كا بسپورى ل عەرەبستانا سعودى

ناڤەروك و ئارمانجێت ڤەكولينێ: ئەڤ ڤەكولينه هاته كرن ب مەرەما رادێ بەلاڤبوونا كەرووا(Fungi) د ناڤ خينا وان كەسێت نڤاندى د ناڤ پشكا چاڤديريا بەردەوام ل نەخوشخانێ بو زانينا ئەوان هوكارێن ترسناك كو د هەمان دەمدا توشى نەخوشى دبن.

24087 بتلێت خينێ ئەوێن دهێنه هنارتن بو پشكێن پزيشكى و باتيبەت پشكا چاڤديريا بەردەوام ل ئێك ژ نەخوشخانێت باژێرێ مەدينا منەوەر ل ولاتێ سعودى هاتنه رەوانەكرن بو تاقيگەها مايكروبايلوجى. دناڤ هەر بتلەكى 5مل بێن خينا نەخوشى تێدا بوون و ئەڤ بتلێن ناڤبرى كرنه د ناڤ ئاميرێ Bac-tec بو ماوێ 7 روژا دا كو گەشه پێ بهێته كرن. ئەو بتلێن كو ئەنجامى وا يێ پوزيتيڤ بوون گەشه پێ هاته كرن لسەر Sabauraud agar و لناڤ پلەيا گەرمايێ30 سەدى هاتنه دانان و جورێت كەروا هاتنه زانين ب رێكا شێوازێ وان هەروەسا ب رێكا بوياغا گرام و *Candida albicans* هاته ديتن ب رێكا Germ tube.

ئەنجام: د ناڤ سەرجەمێ 2875 بتلێن خينێ ئەوێن هاتينه پشكنين كرن 115 بتل كەرو تێدا هاتنه ديتن د ماوێ 7 سالان دا دناڤبەرا 2005 هەتا 2011 . رێژا *Candida albicans* 60بوو(5٪) لێ پا *Candida non-albicans* 53بوو(46٪) وتنێ دوو بتلا *Aspergillus .spp.* تێدابوو ئانكو 2٪ و رێژا مرنێ 32٪ بوو.

ل پشكا شيردانا زاروكا 59 كەس (51٪) كو كەرو د ناڤ خينێ دا هەبوون و ژ وانا 48 (81٪) *Candida albicans* بوون و 11(19٪) *Candida non-albicans* بوون.

ئەو نەخوشێت پتر هەمى كەسا توشى نەخوشيێت كەروا دبن :ئێكەم زاروكێت ژ نى دايكبووين ,و كەسێن توشى تەنكە نەفەسا گران بووين 48٪ , دووەم: بكارئينا 2 يان زێدەتر جورێت دەرمانا و رێژا وان 71٪ بوو.

د پشكا چاڤدێريا بەردەوام بو زەلاما 56 كەسێن توشبووى ب كەروێن ناڤ خينێ دا هەبوون ئانكو (49٪) و ژ وان 12كەس (21٪) *Candida albicans* بوون و 42 (75٪) *Candida non-albicans* بوون و بتنێ 2 كەس (4٪) *Aspergillus .spp.* بوون.

هوكارێن هاريكار بو هندە نەخوشيێن دى د ناڤ پشكێن چاڤدێريا بەردەوام: نەخوشيا شەكرێ 18 كەس بوون(39٪) , بهێن كورتى(تەنگە نەفەس) 9 كەس بوون (19.5٪) ,6 كەس بوون (21٪) پشتى نشتەگەريا, و 3 كەس (6.5٪) ژ هەلگەرانا ئەندامى دەمارى و 3 كەس ژ نەخوشيێت مێلاكێ 2 كەس ژ كێم خينيێ و هايش خونەمانى (گێژبوونێ).

دەرئەنجام: دڤێ ڤەكولينێ دا گرنگيا هەبونا كەروا د ناڤ خينا نەخوشێت نەخوشخانا دياردبيت,رێژا *Candida albicans* 81٪ بوو د ناڤ سەرجەم كەروێن پوزيتيڤ د ناڤ پشكا شيردانێ , هەروەسا رێژا 75٪ يا *Candida non- albicans* بوو د ناڤ سەرجەم كەروێت دناڤ چاڤديريا پشكا بەردەوام بو مەزنا .

الخلاصة

وبائية الفطريات في الدم وعوامل الخطورة المصاحبة للمرضى في مستشفى تخصصي في المملكة العربية السعودية

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.

خلفية واهداف البحث: أعد هذا البحث لمعرفة مدى أنتشار الفطريات في دم المرضى الراقدين في الاقسام العنايةالمركزة من المستشفى لمعرفة العوامل الخطرة المصاحبة.

طرق البحث: تم فحص 24087قنينة دم مرسلة من الاقسام الطبية و خاصةالعناية المركزة منها في مستشفى أحد بالمدينة المنورة في المملكة العربية السعودية الى مختبر مستشفى أحد. كل قنينة فحص كانت تحتوي على5 مل من دم المريض وتم وضع هذه القناني في جهاز ال Bac-tec لتحضين القناني لمدة 7 ايام. قناني الدم الpositive تم زراعتها على Sabauraud agar وتم حضنها بدرجة جرارة 30م وتم التعرف على الفطريات بواسطة اشكالها وكذلك بواسطة صبغة غرام وتم التعرف على ال *Candida albicans* بواسطة Germ Tube.

النتائج: كانت هناك 115قنينة Positive للفطريات من مجموع 2875 قنينة Positive حسب الجهاز لجميع البكتيريا بما فيها الفطرات ل7 سنوات و للفترة من 2005 الى 2011 . نسبة ال*Candida albicans* كانت 60 (52٪) أما ال*Candida non-albicans* فكانت 53 (46٪) وكانت هناك حالتين فقط ل *Aspergillus. spp* أي 2٪ ونسبة الوفيات فكانت 32٪.

في قسم الرضاعة كانت هناك 59 حالة (51٪) فطريات في الدم ومن هذه الحالات 48(81٪) كانت لـ *Candida albicans* و11 (19٪) لـ*Candida non-albicans*.

المرضى الأكثر عرضة للإصابة بالفطريات: أولاً: الأطفال حديثي الولادة, والمصابين بأمراض التنفس العسرة(48٪)

ثانيا: استعمال 2 أو أكثر من المضادات الحيوية وكانت النسبة 71٪ في أقسام العناية المركزة للكبار فكانت هناك 56 حالة فطريات في الدم أي (49٪) و منها 12 حالة (21٪) Candida albicans و42 حالة (75٪) حالة *Candida non-albicans* و فقط حالتين . *Aspergillus. spp*(4٪).

العوامل المصاحبة قي اقسام العناية المركزة: السكري 18 حالة (39٪)، عسر التنفس9حالات (19,5٪) وكذلك 6 حالات (13٪) بعد العمليات. 3 حالات التهاب الجهاز العصبي (5و6٪) وكذلك 3حالات من امراض الكبد, وحالتين لكل من فقر الدم و فقدان الوعي.

الخلاصة: تكشف هذه الدراسة اهمية وجود الفطريات في الدم لمرضى المستشفيات, كانت هناك نسبة 81٪ من ال *Candida albicans* من مجموع الفطريات الpositive من قسم الرضاعة وكانت هناك نسبة 75٪ من الـ*Candida non-albicans* من مجموع الفطريات الpositive من قسم العناية المركزة للكبار.

RELATION OF INFLAMMATORY MARKERS TO SEVERITY AND ACTIVITY OF CHRONIC RHEUMATOID ARTHRITIS

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ABSTRACT

Objectives: Assessment of inflammatory markers High sensitive C-reactive protein (Hs-CRP), Anti-CCP, IL1-alpha, IL - 6) in chronic RA patients and its relation to the disease severity and activity.
Methods: This is a case – control study. The subjects involved in this study were classified into two groups: patients with rheumatoid arthritis (n= 48) and the second group of apparently healthy subjects (n= 30).History and clinical examination were recorded, and then 10 ml of venous blood were obtained from each subject for measurement of (Hs-CRP),(Anti-ccp), (IL-1 alpha), (IL-6).
Results: More than 50% of RA patients included in this study have DAS28 score more than 5.1 and severe form of the disease. The mean disease activity score (DAS28) for RA was (5.6 ± 0.17). The (sever Stanford disability index) was (1.05 ± 0.065). There were highly significant increases in the mean values of serologic and inflammatory biomarkers if RA patients compared with controls: Anti-ccp antibody (18.7 ±1 .89 vs 5.47 ± 0.42 U/ml, $p < 0.001$), serum Hs-CRP (20.59 ± 1.25 vs 4.87 ± 0.52 mg/l, $p < 0.001$), erythrocyte sedimentation rate (ESR) (41.50 ± 2.67 vs 12.07 ± 1.45 mm/hr, $p < 0.001$), serum Interleukin 1-alpha (IL-1 alpha) (50.49 ± 3.61 vs 8.03 ± 0.78 picograms, $p < 0.001$) and Interleukin -6 (IL-6) (86.46 ± 7.33 vs 19.52 ± 1.34 picograms, $p < 0.001$). Patients with high DAS28 (≥ 5.1) and severe disability index have higher mean values of serum Anti-CCP and inflammatory biomarkers levels.
Conclusions: Measurement of serum Anti-ccp antibodies concentration is of high value in the diagnosis of RA. Serum (Hs-CRP, IL-1 alpha, IL-6) can be used as an inflammatory biomarker for evaluating disease activity and severity in RA.

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Key words: Rheumatoid arthritis, inflammatory markers, High sensitive C-reactive protein, Anti-cyclic citrullinated peptide, Interleukin

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by joint pain, swelling, joint tenderness, and destruction of synovial joints, leading to severe disability and premature mortality [1,2] .The disease course can vary widely from mild to aggressive forms, the latter leading to functional loss and increased morbidity and mortality. Modern treatment strategy of RA is early and aggressive use of Disease Modifying Anti-Rheumatic Drug (DMARDs). Whilst the drugs utilized are potentially toxic, it is very important to make a diagnosis as early as possible and to have a prognosis in order to choose the treatment with respect to the expected disease severity [3].

The etiology of RA is still not elucidated. An association between RA and genetic components has been known for many years . Approximately one-third of the genetic contribution is estimated to arise from genes in the human leukocyte antigen (HLA) region, and particularly the HLA-DRB1 alleles that share a similar amino acid sequence, i.e. the "shared epitope"(SE) [4].

Great efforts have been made to identify an infectious agent responsible for

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triggering the disease; however, it has not been possible to isolate any micro-organism from RA-synovial tissue or fluid, and serological analyses have not revealed firm evidence for an underlying infection. Several epidemiological studies have suggested smoking to be a risk factor for RA and rheumatoid factor sero positivity [5].
The disease course in RA is characterized by great inter-individual variation, ranging from mild, self-limiting to severe, erosive disease, sometimes with extra-articular manifestations, such as rheumatoid nodules, pleuritis, pericarditis, and vasculitis and secondary Sjögren syndrome [6].
A simplified and clinically useful version using 28-joint tender and swollen joint counts, (DAS28) has been shown to be a valid measure for disease activity [7].
Tissue injury and inflammatory processes generate a variety of cytokines that alter the metabolic activity of hepatocytes. The pro-inflammatory cytokines Interleukins (IL-1), Tumor necrosis factor (TNFα), and IL-6 in particular increase the production of several plasma proteins such as C-reactive protein (CRP), fibrinogen, α1-antitrypsin, haptoglobin, α1-acid glycoprotein and Serum amyloid A (SAA) protein [8,9].
Also clotting factors, ferritin and some complement components are produced at an accelerated rate, while the synthesis of some other proteins, e.g. albumin, transthyretine and transferrin, is suppressed. The term "the acute-phase plasma protein response" is collectively used for these alterations [10].
The most commonly measured laboratory markers of the acute phase response are the erythrocyte sedimentation rate (ESR) and CRP which are especially high at onset or during the course of disease [11].
Among immunological markers, Rheumatoid Factor (RF) is the most commonly studied and has been associated with a more severe radiological outcome in early RA. The titre of RF at baseline has also been reported to correlate with radiological damage after 3 years of follow-up [11, 12].
More recently the presence of anti cyclic citrullinated peptide antibodies (Anti-CCP) has been demonstrated to be a good predictor of joint erosions in early RA with follow-up periods of 2-10 years [10,12].
The aim of this study was to assess the inflammatory markers (high sensitive C-reactive protein (Hs-CRP), Anti-CCP, IL1-alpha, IL - 6) in chronic RA patients and their relations to the disease severity and activity.

METHODS

A case control stud was adopted to achieve objectives of the study. This study was conducted at the Duhok Center of Rheumatic Disease and Medical Rehabilitation (DCRDMR) from January, 2011 to July, 2011. Seventy- eight individuals including 48 patients with chronic RA (45 females and 3 males) from different areas of Duhok governorate were enrolled in the study. The remainders were 30 control subjects (27 females and 3 males).
Patients fulfilling the 2010 modified American College of Rheumatology (ACR) criteria [13] for RA for at least 12 months were included in the study.
Persons with same age, socioeconomic status and gender as patients, and no history of any rheumatic diseases or autoimmune diseases were included as controls.
A pre-tested questionnaire was designed to obtain information on gender, birth dates, family history of RA, duration of RA, and type of treatment.
Oral consent was obtained from the subjects after the nature of the study had been explained to them. The study protocol was approved by local ethics committee of the Faculty of Medical Sciences – Duhok University.
For purposes of this study, patients were classified according to the duration of disease into three groups: early (< 12

months), intermediate (1 year to 5 years), and long term disease (> 5 years).

The patients were also grouped according to the type of the therapy that they were receiving into those taking only methotrxate and prednisolone, or combination of DMARD.

Using information obtained from the physical examination, laboratory data, and imaging studies, the patient's disease was categorized as mild, moderate, or severe. The patients with mild RA disease had less than six inflamed joints, no extraarticular disease, and no evidence of erosions or cartilage loss on plain radiographs. The patients with severe RA had more than 20 inflamed joints, an elevation in the ESR and/or serum CRP, and one or more of the following:

-Anemia of chronic disease and/or hypoalbuminemia .

-Rheumatoid factor positivity (often in high titer) and/or anti-CCP antibodies.

-Joint radiographs demonstrating rapid appearance of bony erosions and loss of cartilage.

-Extraarticular disease.

The patients with moderate disease, by definition, did not fulfill criteria for either mild or severe disease. They typically had between 6 and 20 inflamed joints and had some combination of the following clinical features:

-Absence of extraarticular disease (most commonly).

-Elevation in the erythrocyte sedimentation rate (ESR) and/or serum C-reactive protein (CRP) concentration.

-Positive RF and/or anti-CCP antibodies.

-Evidence of inflammation on plain radiography, such as osteopenia and/or periarticular swelling; in addition to minimal joint space narrowing and small peripheral erosions.

Disease activity was assessed by using the modified Disease Activity Score derivative for 28 joints (DAS28) [14] .The DAS28 is an index consists of:

1- A 28 tender joint count (range 0-28), a 28 swollen joint count (range 0-28),

2- ESR, and

3- Visual Analogue Scale: An optional general health assessment on a VAS. It was labeled 0=no pain at the left anchor point and 100=severe pain at the right anchor point. The patients were instructed to place a vertical mark on the line to indicate the severity of their pain (Range 0-100).

$$DAS28 = 0.56\times\sqrt{(TJC28)}+0.28\times\sqrt{(SJC28)}+0.70\times\text{lognat(ESR)}+0.014\times GH$$

The level of disease activity (DAS28) can be interpreted as :

Low: DAS28 $\leq$ 3.2

Moderate: 3.2 > DAS28 $\leq$ 5.1, or

High: DAS28 > 5.1

Ten ml of blood was withdrawn from each subject by venipuncture, using plain and EDTA tubes. After 25-30 minutes, the serum was separated by centrifugation at 5000 rpm for 10 minutes. The EDTA blood was used for complete blood counting by using automated hematological analyzer and measuring ESR. The sera were then stored at -28°C for later analysis of other parameters (Anti-ccp, RF, Hs-CRP, IL-I, and IL-6).

Rheumatoid factor (RF) titers were regarded as positive when the level was more than 8 M IU/L. High sensitivity C-Reactive Protein (hs- CRP) was determined by enzyme immunoassay kit (Hs- CRP - ELISA) from Biocheck company. Anti-cyclic citrullinated peptide antibody (Anti-ccp) titers were determined by ASEKULISA ACCP kit (Ref. 3166). Interleukin-6 (IL – 6) levels in serum were measured by Assay Max human IL-6ELISA Kit (cat No. EI1006-1). Interleukin- 1 α (IL - 1 α) levels in serum were measured by Assay Max human IL - 1 α ELISA Kit, (cat No. EI2301-1).

Data were entered into SPSS version 19. Continuous data were expressed as mean ± standard error of the mean (SE). Categorical data were expressed as count and percentages. Differences in the means between three grousp were assessed using one way ANOVA test.

RESULTS

The overall distribution of features are shown in (Table 1). Out of 48 patients, 44 (91.66 %) patients had the disease for more than 2 years. Negative family history of RA was found in 37 (77.1%). Combined therapy (prednisolone and methotraxte) was the treatment in 37 patients (77.1 %). DAS28 of (≥ 5.1) were found in 27 (56.25%). Moderate and severe disease statues was observed in 15 (31.25%), 24 (50%), respectively. Mild Disability on Stanford disability index (DI) was noticed in 27(57.25 %). In addition significant positive latex test, and Anti CCP level of < 18 were found in 43 (89.6 %) and 35(72.9 %), respectively.

X-ray changes of patients with RA are show in (Table 2).

Table 1. Sociodemographic and disease related characteristics of patients with rheumatoid arthritis (No=48).

Demographic Variables		Number	Percentage
Sex	Male	3	6.2
	Female	45	93.8
Duration of disease	≤ 2 years	4	8.3
	2 years ≤ 5	23	47.9
	> 5 years	21	43.8
Type of therapy	Prednisolone and Methtrxate	37	77.1
	Prednisolone , Methtrxate and H ydroxycholoroquin	9	18.8
	Azathioprine and Prednisolone	2	4.2
Family History of RA	Negative Family History of RA	37	77.1
	Positive family History of RA	11	22.9
DAS28	≤ 3.1	3	6.25
	3.2 - 5.1	18	37.5
	≥ 5.1	27	56.25
Severity of Disease	Mild	9	18.75
	Moderate	15	31.25
	Severe	24	50.0
Stanford disability index(DI)	Mild disability	27	56.25
	Moderate disability	21	43.75
	Severe disability	1	0.21
Latex test	Negative Latex test	5	10.4
	Positive Latex test	43	89.6
Anti-CCP	ACCP ≤ 12	23	47.9
	ACCP 12 – 18	12	25.0
	ACCP ≥ 18	13	27.1

Table 2. X-ray Changes in patients with rheumatoid arthritis.

X-ray Changes		Feet X-ray	Hand X-ray	Cervical X-ray
Early Changes of RA	No.	28	32	42
	%	58.3	66.7	87.5
Late Changes of RA	No.	20	16	6
	%	41.7	33.3	12.5
Total No. of patients			48	

Twenty patients (41.7 %) had late changes in the feet x-ray and only 12.5 % (n= 6) had late X-ray changes of the cervical spine, while early changes of RA were seen in feet, hand and cervical X-ray 58.3 % (n=28), 66.7% (n=32) and 87.5 % (n=42) respectively. Early changes were indicated by osteopenia and/or periarticular swelling in addition to minimal joint space narrowing. However, late changes indicated by presence of bone erosions.

(Table 3) shows type of therapy in relation to DAS28 in patients with rheumatoid arthritis. Most of the patients were on prednisolone and methotraxte (37), 24 of them had DAS28 (3.2 – 5.1).

Table 3. Type of therapy in relation to DAS among patients.

Type of therapy	DAS28			Total
	< 3.1	3.2 - 5.1	> 5.1	
Prednisolone and Methotrxate	2	24	11	37
Prednisolone, Methotrxate and Hydroxycholoroquin	1	4	4	9
Azathioprine and Prednisolone	0	1	1	2

Rheumatoid arthritis patients revealed significant differences in serum concentrations of serological biomarkers and hematological parameters compared with controls: Anti-CCP (18.7 ± 1.89 vs 5.47 ± 0.42 $p < 0.001$) , IL- 6 (86.46 ± 7.33 vs 19.52 ± 1.34 ,$P < 0.001$) , IL-1 alpha (50.49 ± 3.61 vs 8.03 ± 0.78 , $P < 0.001$), Hs-CRP (20.59 ± 1.25 vs 4.87 ± 0.52, $P < 0.001$), ESR (41.50 ± 2.67 vs 12.07 ± 1.45, $P < 0.001$), and Hb (12.24 ± 0.18 vs 13.07 ± 0.24, $P < 0.01$). However, no significant differences were seen in both WBC count and platelets count (Table 4).

Table 4. Differences in the serological biomarkers and hematological parameters between patients with rheumatoid arthritis and controls (* Mean ± SE).

Variables	Patients with RA *	Control *	P value
Anti –CCP	18.7 ± 1.89	5.47 ± 0.42	< 0.001
IL- 6	86.46 ± 7.33	19.52 ± 1.34	< 0.001
IL-1 alpha	50.49 ± 3.61	8.03 ± 0.78	< 0.001
Hs-CRP	20.59 ± 1.25	4.87 ± 0.52	< 0.001
WBC	8.19 ± 0.29	7.76 ± 0.73	NS
Hb	12.24 ± 0.18	13.07 ± 0.24	< 0.01
HCT	35.44 ± 0.81	38.77 ± 0.76	< 0.01
Plat	279.72 ± 12.71	250.50 ± 10.51	NS
ESR	41.50 ± 2.67	12.07 ± 1.45	< 0.001

* *Mean ± SE*

(Table 5) reveals the serological biomarkers according to DAS28 levels in RA patients. It shows higher values of Anti –CCP, Hs-CRP and IL-6 at higher DAS28 levels (DAS28≥5.1).

Distribution of related risk factors in patients group in relation to severity of disease is revealed in (Table 6), which demonstrates that 50 % (n= 24) of patients had severe disease form. Of them 62.5 %

(n=15) had DAS > 5.1. Anti-CCP of > 12 was found in 66.66 % (n=16), positive latex test was found in 87.5 % (n=21) and Stanford DI of moderate type was found in 54.1 % (n=13).

Table 5. Serological biomarkers according to DAS28 level in patients with RA.

Variables	DAS28 ≤ 3.1 6.25% (n= 3)	DAS28 3.2 - 5.1 37.5 % (n=18)	DAS28 ≥ 5.1 56.25% (n=27)	Mean ± SE
ACCP		< 3.1		12.35 ± 0.41
		3.2 - 5.1		18.29 ± 2.57
		> 5.1		20.49 ± 3.22
Hs-CRP		< 3.1		22.44 ± 6.48
		3.2 - 5.1		18.68 ± 1.72
		> 5.1		23.15 ± 1.75
IL- 6		< 3.1		116.48 ± 38.76
		3.2 - 5.1		72.64 ± 6.17
		> 5.1		102.17 ± 15.45
IL-1 alpha		< 3.1		67.65 ± 17.58
		3.2 - 5.1		45.33 ± 4.25
		> 5.1		55.38 ± 6.47

Table 6. Distribution of related risk factors in patients group in relation to severity of disease.

Variables		Severity of Diseases Mild	Moderate	Severe
DAS28	< 3.1	1	0	2
	3.2 – 5.1	7	13	7
	> 5.1	1	2	15
Anti CCP Level	< 12	5	9	8
	> 12 < 18	2	4	7
	> 18	2	2	9
Latex test	Negative	0	2	3
	Positive	9	13	21
Stanford disability Index	Mild DI	6	10	11
	Moderate DI	3	5	13
	Severe DI	0	0	1

The distribution of DAS28, Stanford DI, serological and hematological parameters according to severity of disease in patients group is shown in (Table 7). Fifty percent of them had severe disease pattern, which showed high score of DAS28, Stanford DI, and increases in the concentrations of Anti –CCP , Hs-CRP, IL-6 , and ESR. Despite that some of them were statistically significant as demonstrated in (Table 8), by one way ANOVA analyses.

Multivariate analysis for both groups using different levels of Anti-CCP and both serological biomarkers and hematological parameters as depending variables are revealed in (Table 9). It shows different degrees of significance from statistical point of view for all groups.

Table 7. Distribution of Serological and Hematological Parameters according Severity of Disease level in patients group (n= 48).

Variables	Severity of Disease Mild 18.75 % (n= 9)	 Moderate 31.25 % (n=15)	 Severe 50.0% (n=24)
	Mean ± SE	Mean ± SE	Mean ± SE
DAS28	3.81 ± 0.4	4.76 ± 0.19	5.6 ± 0.17
Stanford Disability Index	0.90 ± 0.21	0.94 ± 0.09	1.05 ± 0.065
Anti – CCP	12.48 ± 0.85	13.67 ± 1.89	24.47 ± 3.24
Hs-CRP	23.32 ± 3.769	16.73 ± 2.03	23.07 ± 1.54
IL – 6	95.44 ± 17.78	76.70 ± 10.09	92.17 ± 12.08
IL – 1 alpha	62.53 ± 8.10	45.08 ± 5.99	51.83 ± 5.20
ESR	41.33 ± 4.79	38.89 ± 4.26	43.70 ± 4.19
WBC	8.00 ± 0.77	8.72 ± 0.46	7.79 ± 0.42
Hb	12.80 ± 0.51	12.31 ± 0.34	12.02 ± 0.23

Table 8. One way ANOVA analysis between Serological Biomarkers and Hematological Parameters of patients group and Severity of Disease.

Dependent Variables	Severity of Disease	Severity of Disease Between Group	P value
DAS28	Mild Disease	Moderate Disease	NS
		Sever Disease	0.005
	Moderate Dieses	Mild Disease	NS
		Sever Disease	0.002
	Severe Disease	Mild Disease	0.005
		Moderate Disease	0.002
Stanford Stability Index	Mild Disease	Moderate Disease	NS
		Sever Disease	NS
	Moderate Disease	Mild Disease	NS
		Sever Disease	NS
	Severe Disease	Mild Disease	NS
		Moderate Disease	NS
ACCP	Mild Disease	Moderate Disease	NS
		Sever Disease	NS
	Moderate Dieses	Mild Disease	NS
		Sever Disease	NS
	Severe Disease	Mild Disease	NS
		Moderate Dieses	NS
	Moderate Dieses	Sever Disease	NS
		Mild Disease	NS
		Sever Disease	NS
	Severe Disease	Mild Disease	NS
		Moderate Disease	NS
		Moderate Disease	NS

IL- 6	Mild Disease	Sever Disease	0.003
		Mild Disease	NS
	Moderate Dieses	Sever Disease	NS
		Mild Disease	0.003
	Severe Disease	Moderate Disease	NS
		Moderate Disease	NS
IL-1	Mild Disease	Sever Disease	0.001
		Mild Disease	0.049
	Moderate Dieses	Sever Disease	NS
		Mild Disease	NS
	Severe Disease	Moderate Disease	NS
		Moderate Disease	NS
WBC	Mild Disease	Sever Disease	NS
		Mild Disease	NS
	Moderate Dieses	Sever Disease	NS
		Mild Disease	NS
	Severe Disease	Moderate Disease	NS
		Moderate Disease	0.003
Hb	Mild Disease	Sever Disease	0.001
		Mild Disease	0.003
	Moderate Dieses	Sever Disease	NS
		Mild Disease	NS
	Severe Disease	Moderate Disease	NS
		Moderate Disease	NS
ESR	Mild Disease	Sever Disease	0.001
		Mild Disease	NS
	Moderate Dieses	Sever Disease	NS
		Mild Disease	0.001
	Severe Disease	Moderate Disease	0.029

Significant value P < 0.05 NS Not significant

Table 9. One way ANOVA analysis between Serological Biomarkers and Hematological Parameters of patients and Controls with different Anti-CCP levels.

Dependent Variables	Anti CCP Group	Anti CCP Group	Mean Difference	P value
Hs-CRP	< 12	12 - 18	-12.46*	< 0.001
		> 18	-12.71*	< 0.001
	12 – 18	< 12	12.46*	< 0.001
		> 18	-.25	NS
	> 18	< 12	12.71*	< 0.001
		12 - 18	.252	NS
IL- 6	< 12	12 - 18	-62.73*	< 0.001
		> 18	-30.64	NS
	12 – 18	< 12	62.73*	< 0.001
		> 18	32.09	NS
	> 18	< 12	30.64	NS
		12 - 18	-32.10	NS
IL-1	< 12	12 - 18	-26.85*	0.007
		> 18	-21.31*	0.035
	12 – 18	< 12	26.85*	0.007
		> 18	5.54	NS
	> 18	< 12	21.31*	0.035
		12 - 18	-5.54	NS

ESR	< 12	12 - 18	-24.62*	< 0.001
		> 18	-15.59*	0.027
	12 – 18	< 12	24.62*	< 0.001
		> 18	9.03	NS
	> 18	< 12	15.59*	< 0.001
		12 - 18	-9.03	NS

** Statistically significant*

DISCUSSION

Studies in recent years were conducted for evaluation of inflammatory biomarkers including Anti-CCP, Hs-CRP and cytokines such as IL1-alpha and IL- 6 in rheumatologic disorders including rheumatoid arthritis, in order to assess their role in pathogenesis, diagnosis, severity of RA and to make them prognostic tools for patients with this disabling disorder, and also to find new treatment modality based on their role in pathogenesis of RA [15].

Findings of the present study showed that concentrations of the inflammatory biomarkers were significantly increased in patients with chronic RA, when compared to healthy control group.

In addition, our results suggest that not only the traditional inflammatory biomarkers and routine hematological investigations give clue to the diagnosis but also there is evidence of IL-1- alpha and IL-6 in plasma may be significant. The importance of these inflammatory biomarkers and other demographic factors (DAS28 and Stanford DI) is a matter of controversy [14].

Results of the present study show that there is a high percentage of sever disease status. Fifty percent of the patients had severe disease form. Of them about 62.5 % had DAS > 5.1 and 67 % had Anti-CCP of > 12. Positive latex test was found in approximately 87 %.

Few studies have examined severity of rheumatoid arthritis in relation to disability by using new measurement, such as HAQ and DAS28 in relation to inflammatory biomarkers in Iraq [15]. As far as we are aware, no previous study has examined the frequency of Anti-CCP, IL-1 alpha and IL-6 in patients with RA and assessed their role in relation to disease activity by using DAS in this locality.

Both severity and activity of RA were correlated with inflammatory biomarkers in the patients. In this study, associations between severity of disease, DAS28, IL-1-apha, IL-6, Hb and ESR were observed.

The disease pattern and joint distribution in our patients are similar to those in Europe but the disease appears to be generally less destructive. Severe systemic upset and extra-articular manifestations appear to be less common in our population [16]. Several drug treatments using intensive combination treatment regime with or without steroids were given to our patients. Most of them were on prednisolone and methotraxte (37%). They had DAS (3.2–5.1) (moderate disease activity). Moreover, in observational cohorts, where conventional DMARDS have been used according to their physicians, choice in a routine clinical setting, have reported rates of remission varying between 7 and 30%.

A prospective, longitudinal study of 142 patients with early RA (< 2years) with a mean follow-up of 6 years, treated by strategy using traditional DMARDS and steroids showed that 20%, 27% and 32% of the patients achieved ACR remission at 1st year, 2nd year and in the last visit respectively [17].

While in this study no one achieved remission with DAS < 2.6 and 50% of them had DAS ≥ 5.1. At the same time, they showed an increase in the concentrations and value of Anti –CCP, Hs-CRP, IL-6, and ESR and decrease in the level of Hb. However, they were statistically not significant.

In the present study, the patients revealed significant differences in serum concentrations of serological biomarkers and hematological parameters. These results are in agreement with those of the previous studies that have uniformly reported greater concentrations of Anti-CCP, Hs-CRP, IL-1- alpha, IL-6 and ESR with low Hb in the patients with chronic RA [18].

The high prevalence of Anti-CCP in RA patients with extensive disease activity and severe radiological changes, and even in RA patients, who are IgM-RF-negative, suggests that Anti-CCP is more useful than the RF in the early prediction of disease outcome and disease activity.

Significant differences exist in the serological biomarkers and hematological parameters among the different groups of Anti-CCP. These findings were also observed in other studies [19]. An important point concerning the diagnostic value of Anti-CCP is that about 35–40% of the RF-negative RA patients score positively for Anti-CCP.

The value of Anti-CCP antibodies and RF for predicting the outcome of RA, clinical signs of disease activity, and the severity of radiographic joint damage have been investigated recently. Several studies support the hypothesis that RA patients with positive Anti-CCP, develop significantly more radiological damage than Anti-CCP negative patients [12,20] .

In this study, the early radiological changes of cervical spine were found in 42 patients mainly due to osteoarthritis changes probably related to their occupation. About 69 % of the Anti-CCP positive patients had late radiological changes in hands compared to only 31 % of Anti-CCP negative patients. Several studies in recent years have been conducted to confirm the role of inflammatory cytokines (IL-1-alpha and IL-6) in the pathogenesis of rheumatoid arthritis [18,20].

The level of IL-1-alpha was significantly higher in patients when compared with the healthy control. Furthermore, it was also statistically significantly higher in RA group with moderate DAS28 when compared to RA patients with mild DAS28. Despite the fact that these data are in agreement with results of what has been reported in the literature[12], few other studies have also found either weaker or significant correlations of IL-1-alpha with severity and activity of the disease [21]. The role of IL-6 in the pathogenesis of RA is also controversial. While some authors found elevated IL-6 levels only in cases with increased CRP, concluding that it is part of the acute phase response [22]. The results of our study are in agreement with most of these reports, since IL-6 level is significantly increased in patients with RA when compared to the healthy control patients. IL-6 was also significantly higher in RA patients with severe disease form and in those with high DAS28 compared to RA group with mild DAS.

Serum IL-1-alpha and IL-6 were sensitive markers for RA disease activity. They may be useful independent markers for prediction of RA disease activity and to differentiate normal subjects from those having RA. More studies should be done in this field for better evaluation of RA patients covering all aspects of the disease like clinical features, complications, treatment modalities in Kurdistan. Further studies are recommended to evaluate the role of both IL-1-alpha and IL-6 in pathogenesis of RA and their possible therapeutic implications in the treatment of RA.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest

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پوختە

هەوکردنی جومگەی روماتیزمی (RA) , نەخوشییەکی هەوکردنی درێژگایەنە دەناسرێتەوە بە ئەستوربوون وئازاری جومگەولە کوتایی تێکشکانی جومگە (پەردەی جومگە)

سەرەرای ئەوی هۆی راستەوخۆی تووش بوونی هەوکردنی جومگەی روماتیزمی بەتەواوی نەزانراوە , بەلام بەڵگەی بەهێزهەیە لەسەر بوونی دژەبەرگری خۆیەتی کەزۆریك لە دژەتەنەکای پەیوەندیان هەیە لە کەڵ نەخۆشیەکە , گرنگترینیان بریتیە لە دژەتەن دژبە (anti-ccp antibodies, Hs-CRP , Il-6 , Il-1 alpha) سی ریئاکتیف پروتێن (CRP) یەکێکە لە بنەرەتێرین زوو وەلام دانەوەی بەرگری لەش دژبە هەوکردن .

ئامانج:بو پشکنین پەیوەندی نێوان نیشانەکانی کلینکی و پەککەوتن پیشەیی

بو بیشکنین پەیوەندی نێوان نیشانەکانی تاقیگەین (سیرۆلۆجی وهیماتۆلۆجی) وپەککەوتنی پیشەیی

ڕێگا :شێوازی ئەم لێکولینەوە لە نێوان نەخۆش وبەراوردکردنی بە کەس ئاساییە , پۆلێن کردنی کەسانی بەشدار بوو لەم لێکۆلینەوە بۆ دوو گروب دابەش کراوە:

گروپی یەکەم بریتی بوون لە 48 نەخۆش تووشبوو بە هەوکردنی جومگەی روماتیزمی.

گروپی دووەم بریتی بوون لە 30کەسانی ئاساییی . پاش وەرگرتنی مێژووی نەخۆش وهەڵسەنگاندنی کلینیکی , بری 10مل خۆین لە هەریەك لە کەسەکان وەرگیرا بۆ ئە نە نجامدانی پیشکنینی

(Il-6 , Il-1 alpha , Anti-ccp , Hs-CRP , ESR) شیوازی ئیلایزا بەکارهاتووە بۆ پیشکنینی ڕێژەی , (Il-6 , Il-1 alpha , Anti-ccp , Hs-CRP)

بە کەرەستەی تابیەت .

ئەنجام :تێکرای چالاکی نەخۆش هەوکردنی جومگەی روماتیزمی (Sever DAS28 score) (0.17 _+5.6) و تێکرای پەککەوتنی پیشەیی بەڕێگای (Sever Stanford disability index) (0.065 _+1.05).

بەرز بوونە وەیکی گرینگ لە تێکرای ڕێژەی سیرەمی (Anti-ccp) (1.89 _+18.7 بەرامبەر 0.42 _+5.47ی /مل , P<0.001) و (Hs-CRP) (1.25 _+20.59بەرامبەر 0.52 _+4.87ملغ / ل , P<0.001) و (ESR)(_+41.50 2.67بەرامبەر 1.45 _+12.07مم/ 1سعات , P<0.001) و(IL-1 alpha)(3.61 _+50.49بەرامبەر 0.78 _+8.03 بیکو غرام ,P<0.001) و (IL-6)(7.33 _+86.46بەرامبەر 1.34 _+19.52بیکوغرام , P<0.001)

لە نێو نەخۆشەکان بەدیکرا بە بەراورد لە گەل کەسە ئاساییەکان.

دەرئەنجام:پێوانی ڕێژەی سیرەمی (Anti-ccp antibodies) بەهایەکی کلینیگی هەیە بۆ دەست نیشان کردنی نەخۆش هەوکردنی جومگەی روماتیزمی.

بەکار هێنانی ڕێژەی سیرەمی (IL-6 , IL-1alpha , Hs-CRP) بە دانانی یەکێکی لە نیشانە بایلوجی بۆ هەڵسەندگاندنی چالاکی نەخۆش هەوکردنی جومگەی روماتیزمی.

الخلاصة

العلاقة بين علامات للالتهابات مع شدة ونشاط التهاب المفاصل الروماتويدي المزمن

التهاب المفاصل الرثوي (RA) هو مرض التهابي مزمن يتميز بتورم المفصل والآلام المفصل عند الضغط , ويسبب تدمير المفصل ألزلالي. على الرغم من إن المسببات الدقيقة لالتهاب المفاصل الرثوي مازال مجهولا , وهناك أدلة قوية عن دور المناعة الذاتية بواسطة الأجسام المضادة لها ارتباط بهذا المرض , ولكن من أهمها (anti-ccp antibodies, Hs-CRP , Il-6 , Il-1 alpha) . (CRP) هي واحدة من أهم الاستجابات واقرب إلى إصابة التهابية .

الهدف:. التحقيق في وجود علاقة بين النتائج السريرية ودرجة العوق عندا مرضى الرثية المفصلية .

. التحقيق في وجود علاقة بين النتائج الهيماتولوجية والسيرولوجية ودرجة العوق عند مرضى الرثية المفصلية.

المواد وطريقة العمل:تم في هذا البحث دراسة الحالات والشواهد السريرية لمرضى التهاب المفاصل الرثوي حيث تم تصنيف الأشخاص المشاركين في هذه الدراسة إلى مجموعتين

المرضى الذين يعانون من التهاب المفاصل الرثوي (ع=48)

والمجموعة الثانية من الأشخاص أصحاء (ع= 30) كمجموعة ضابطة .

وتم تسجيل تاريخ المرض وإجراء الفحص السريري , واخذ عينة الدم الوريدي (10مل) من المجموعتين وذلك لقياس (serum Il-6) , (serum Il-1 alpha),(serum Anti-ccp), (serum Hs-CRP

النتائج:كان درجة نشاط المرضى (Sever DAS28 score) (5.6+_ 0.17) وكان نتيجة العجز وظيفي (Sever Stanford disability index) (1.05+_ 0.065)

من نتائج الفحوصات المختبرية وجد هنالك زيادة معنوية في قيم تركيز (Anti-ccp) (18.7+_ 1.89 مقابل 5.47+_ 0.42 u/مل , P<0.001) و (Hs-CRP)(20.59+_ 1.25 مقابل 4.87+_ 0.52 ملغم / لتر , P<0.001) و(ESR)(41.50+_ 2.67مقابل 12.07+_ 1.45مم/ 1ساعة , P<0.001) و (IL-1 alpha) (50.49+_ 3.61 مقابل 8.03+_ 0.78 بيكو غرام ,P<0.001) و (IL-6)(86.46+_7.33 مقابل 19.52+_ 1.34بيكوغرام , P<0.001) في المجموعة المرضى بالمقارنة مع الأشخاص الأصحاء .

الاستنتاجات:قياس تركيز (Anti-ccp antibodies) ذات قيمة سريرية مهمة في تشخيص التهاب المفاصل الرثوي

ويمكن استخدام تركيز (IL-6 , IL-1alpha , Hs-CRP) في المصل باعتبارها العلامات البيولوجية لتقييم نشاط المرض في التهاب المفاصل الرثوي .

THE DETECTION OF TUMOR AND INFLAMMATORY ANGIOGENIC STIMULATIONVIA VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) EXPRESSION IN RELATION TO MICROVESSEL DENSITY (MVD) IN ORAL SQUAMOUS CELL CARCINOMA

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ABSTRACT

Background: Tumor growth is angiogenesis-dependent process that is mediated by growth factors such as vascular endothelial growth factor
(VEGF). The main goal of this study is to detect VEGF expression by tumor cells and tumor -associated inflammatory cells to be correlated with microvessel density (MvD) in Oral squamous cell carcinomas.
Materials and Methods: The study included 30 Paraffin embedded tissue blocks that are diagnosed as oral squamous cell carcinoma. An immunohistochemical staining with anti VEGF and anti CD34 antibodies were performed.
Results: VEGF expression was moderately correlated with tumor site (r=0.34), highly correlated with tumor size and histopathological grade (r=0.97, r=0.71) respectively, and weakly correlated with lymph node involvement (r=0.25).
There was moderate -strong correlation between VEGF expression in both tumor cells and inflammatory cells (r=0.69).
MvD showed a strong correlation with tumor size, tumor site and moderate correlation with histopathological grading. A highly significant difference was found in MvD with VEGF expression, but, in a weak correlation between them.
There was a very weak correlation of VEGF expression in inflammatory cells with MvD .
Conclusion: VEGF upregulation is correlated to tumor angiogenesis. In addition, both tumor cells and inflammatory cells namely macrophages, were identified as contributors for VEGF expression in the tissue.

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Key words: Tumor, VEGF, MVD, Oral Squamous Cell Carcinoma….

Tumor angiogenesis is mediated by growth factors produced by tumor cells and/or by tumor-infiltrating inflammatory cells, VEGF is the leading candidate of these factors[1,2]. Inflammation may be responsible for a substantial portion of tumor vascularization in what is called ‘‘inflammatory angiogenesis,’’ [3].
The aim of this study is to Immunohistochemically detect VEGF expression by tumor cells and tumor -associated inflammatory cells to be correlated with microvessel density (MvD) and to correlate both with various clinicopathological features in oral squamous cell carcinomas.

METHODS

Tissue Samples of thirty paraffine-embeded blocks of oral squamous cell carcinoma (OSCC) were obtained fromDepartment of Oral Pathology, College of Dentistry, University of Baghdad, from private histopathological lab, from 1999-2007. The clinico-pathological characteristics of the selected

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specimens are shown in (Table 1) All samples were fixed in 10% formalin and processed to provide paraffin blocks. Staining with heamatoxylin and eosin was carried out on the 5µm section from each block for histopathological examination and to define the tumor grade: well, moderately or poorly differentiated [4]. All the 4µm tissue sections of the collected specimens were subjected to Immunohistochemical protocols. Briefly, the sections were dewaxed in xylene, rehydrated, antigen retrieval was performed by incubation in buffer citrate (pH 8) in water bath, and then the sections incubated with hydrogen peroxide. After incubation with normal serum, the slides were incubated with anti-CD34and anti-VEGF (US biological) antibodies. Antibody binding was localized using a secondary biotinlated antibody and the sterptavidin-conjucated horseradish peroxidase (DAKO, Denmark), and revealed using DAB chromogenic substrate for peroxidase. After counterstaining with Mayer's hematoxylin, the slides then dehydrated and mounted with DPX. they showed well preserved tumor islands with a good IHC staining) for VEGF antibodies. The evaluation and scoring of the positive immunostaining of VEGF was performed at 400X magnification. Positive VEGF immunostained inflammatory cells within the same fields were counted.

According to De Cicco et al (2004) [6] , the Evaluation of immunohistochemical expression of VEGF was based on semiquantitative evaluation of percentage of positive cells for VEGF{0(negative), 0% positive cells; 1(mild), <25% positive cells; 2(moderate), 26-50% positive cells and 3(strong),>50% positive cells} ,in addition to that staining intensity(0,negative;1,weak; 2,intermediate and 3,strong), where the staining intensity was similar to that of endothelial cells, the specimen was determined as intermediate, staining that was weaker or stronger than endothelial cells in the specimen was determined as weak or strong, respectively.

Evaluation of inflammation: It was performed in accordance with the method proposed by Offersen et al (2002) [7] .A semiquatitative scale to assess the degree of inflammation was established, and inflammation was scored as: Minimal inflammation, less than 5 mononuclear inflammatory cells within 10x10 grid at x200, Moderate inflammation, mononuclear inflammatory cells scattered throughout the tissue, but background stromal connective tissue still clearly visible ,and, Intense inflammation: mononuclear inflammatory cells densely infiltrate the tissue lying side by side.

No evaluation was performed in areas of necrosis.

Table 1. Clinicopathological characteristics of 30 cases with oral squamous cell carcinoma.

	NO.	%
Age		
<50	**10**	**33.34**
>50	**20**	**66.66**
Gender		
Female	**9**	**30**
Male	**21**	**70**
Tumor Site		
Tongue	**13**	**43.34**
Floor of mouth	**5**	**16.66**
Buccal mucosa	**6**	**20**
Labial mucosa	**6**	**20**
Tumor size		
T1	**6**	**20**
T2	**6**	**20**
T3	**5**	**16.66**
T4	**13**	**43.34**
Lymph Node		
Negative	**19**	**63.33**
Positive	**11**	**36.66**
Histological Grade		
Well	**9**	**30**
Moderate	**15**	**50**
Poor	**6**	**20**

Statistical Analysis: Statistical analyses were computer assisted using SPSS version (12) (SPSS professional 2005). The parametric statistical tests were used namely two –tailed T-test and ANOVAs table. The non-parametric statistical tests were used namely Chi- square test. P-values less than (0.05) level of significance was considered statistically significant at 95% confidence. The statistical correlations were measured by Pearson's and Spearman's linear correlation coefficient

RESULTS

The study sample consisted of 21 males(70%) and females (30%), with a mean age (54.13) years. The most affected site was the tongue(43%). The clinical staging revealed that, 43% were found in T4, and 37% with lymph node involvement. Histopathological grading showed that ,50%were moderately differentiated OSCC and 80% expressed heavy infiltration by inflammatory cells which mainly lymphocytes in addition to plasma cells and macrophages. Whereas only 20% (6 cases) were moderately infiltrated by inflammatory cells (Figure 1).

A strong correlations were recorded between the increase in the intensity of tumor-associated inflammatory cells infiltration and each of Tumor size (r=0.7), lymph node metastasis (r=0.98) and with the histopathological grade (r=0.94).

Vascular Endothelial Growth Factor (VEGF) Expression: From all 30 OSCC specimens, 53.33% (16 cases) show moderate positive expression for VEGF, while 46.66 % (14 cases) show strong positivity. VEGF staining intensity was strong in 63 % (19 cases), and moderate in 37 % (11 cases) (Figure 2).

Infiltrating inflammatory cells in the tumors were stained positively for VEGF in only 20 % (6 cases) out of 30 OSCC cases.

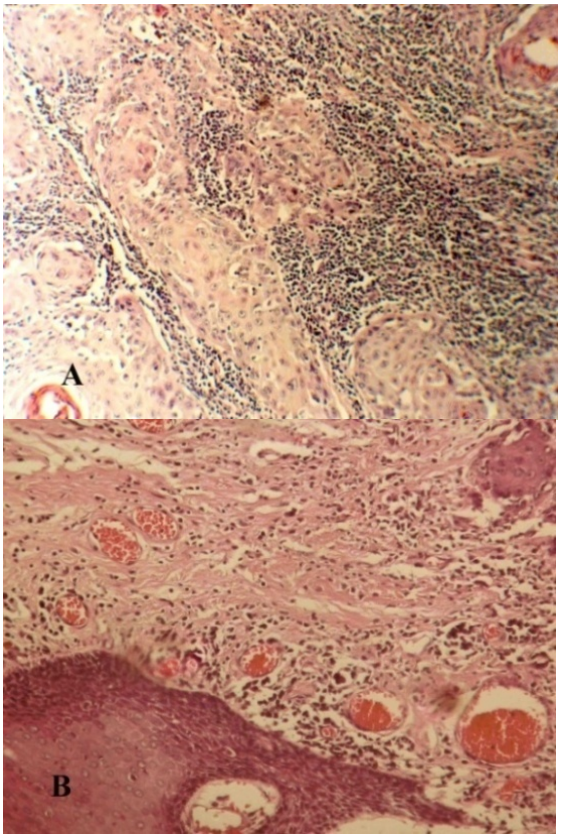

Figure 1. Oral squamous cell carcinoma (A) heavy infiltration by inflammatory cells (B) moderate inflammatory cells infiltration(X100).

Macrophages were the most predominant type of inflammatory cells in these positive specimens that show positive staining for VEGF in compare with few lymphocytes with positive staining for VEGF (Figure 3).

Immunohistochemical staining results, indicated that, VEGF is upregulated in oral squamous cell carcinoma confirmed by the statistically significant difference in vascular VEGF expression between the normal oral mucosa and the tumor cells (p=0.01). There were statistically significant differences recorded in vascular VEGF expression among different tumor sites, tumor sizes, lymph node involvement, and the histopathological grades (p=0.03, p=0.000, p=0.000, p=0.03) respectively.

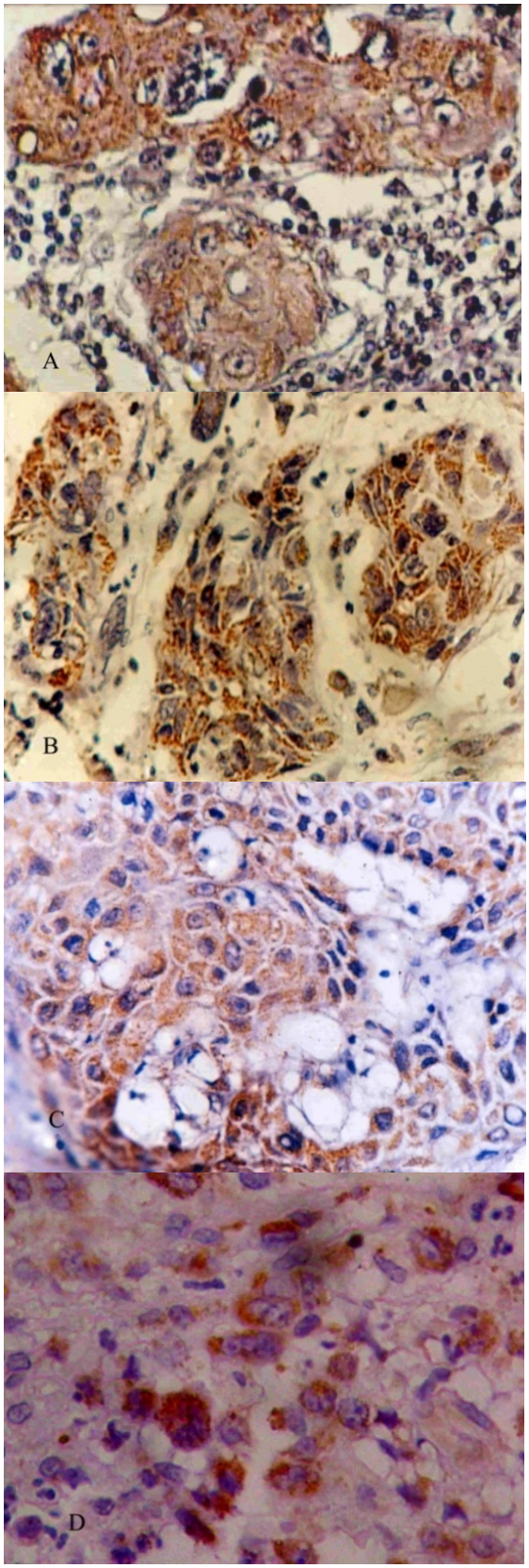

Figure 2. positive VEGF expression in oral squamous cell carcinoma A,B(Stong) ,C,D (Modeate) (X400).

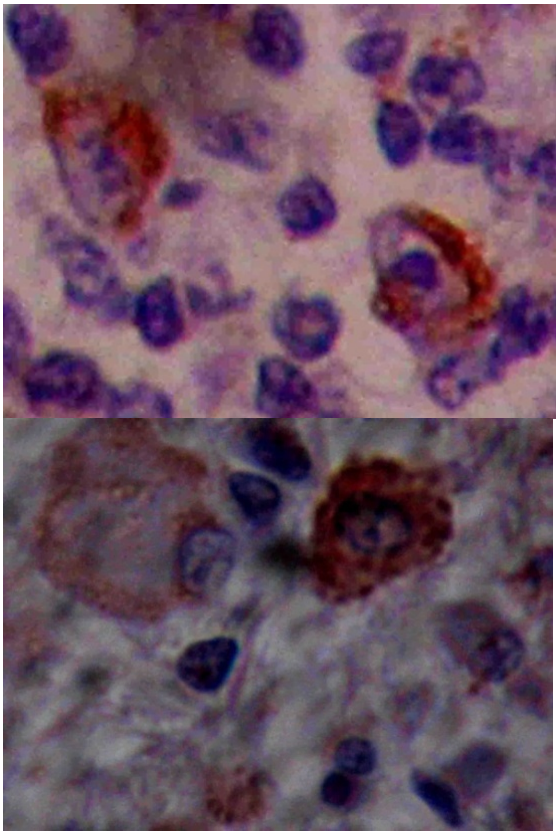

Figure 3. VEGF positive expression in Macrophages associated with OSCC (arrows) (X1000).

Moreover, VEGF expression was moderately correlated with tumor site (r=0.34), highly correlated with tumor size and histopathological grade (r=0.97, r=0.71) respectively, and weakly correlated with lymph node involvement (r=0.25).

Highly significant statistical differences (p=0.000)and strong correlations(r=0.69) were recorded between VEGF expressions in tumors in which the infiltrating inflammatory cells positively expressed VEGF and those tumors with infiltrating inflammatory cells negatively expressed VEGF.

Microvessel density assessed by CD34 immunostaining: CD34 Immunostaining was detected as brown cytoplasmic endothelial cells in both controls and tumor samples (Figure 4).

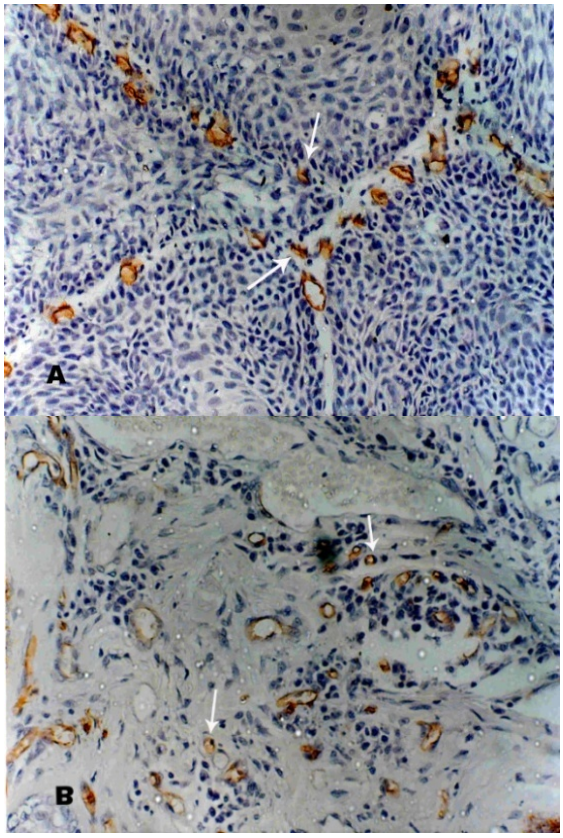

Figure 4. (A) Intra tumoral Microvessel densities, (B) Peri tumoral Microvessel densities (arrows) (X200).

The mean MvD in normal oral mucosa was (19.5) .The mean count of the tumor microvessels (intra-tumoral and peri-tumoral MvD) was (29.40 and 33.93) respectively. (Figure 5).

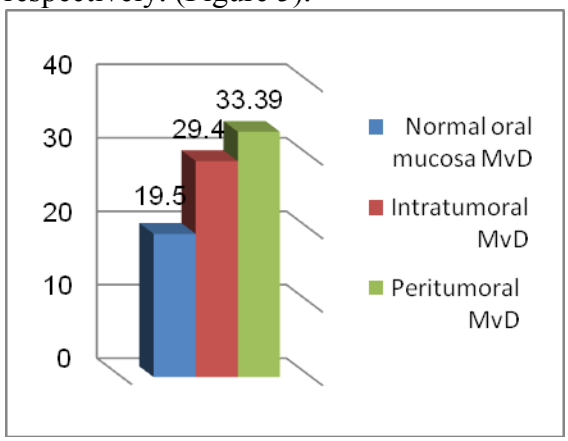

Figure 5. The mean count of normal oral mucosa MvD and tumor MvD in normal oral mucosa specimens and OSCC cases.

In spite of there was no statistically significant difference in MvD concerning the tumor site, tumor size and histopathological grades, MvD showed a strong correlation with tumor size, tumor site(r=0.99,r=0.75) respectively, and moderate correlation with histopathological grading(r=0.56). There was a high significant difference in MvD between positive and negative lymph node involvement (p=0.000), but there was no correlation detected between them. A highly significant difference was found in MvD regarding the intensity of inflammation. Additionally, MvD increases with the increasing intensity of inflammation, indicated by the moderate to weak correlation between peri-tumoral MvD and the intensity of inflammation (r=0.24) and also between Intra –tumoral MvD and degree of inflammation (r=0.19). There were highly significant differences in MvD with different degrees of VEGF expression (in both positivity and intensity), but, in a weak correlation in VEGF Expression and MvD. Similarly, MvD in tumors in which the inflammatory cells where positive for VEGF was significantly increased than those tumors with negative expression of inflammatory cells for VEGF (p=0.00), but a weak correlation was found in this context in OSCC sample.

DISCUSSION

Angiogenesis, which is regulated by a number of angiogenic factors, plays a crucial role in solid tumor growth and facilitates tumor progression and metastasis [8,9].

Several studies reported a non- significant difference in VEGF expression among different tumor sizes [10-13]. The present study revealed a statistically significant difference in VEGF expression among different tumor sizes. This result was confirmed by the strong correlation between VEGF expression and size of tumor. This may be due to that tumors cannot enlarge beyond 1–2mm3 in diameters or thickness unless they are vascularized; in addition, angiogenesis

plays a crucial role in solid tumor growth and facilitates tumor growth and metastasis [14,15..]

VEGF expression in OSCC specimens with lymph node metastasis was significantly higher than those in the absence of lymph node metastasis, but in a weak correlation. However, the capability of VEGF to increase vascular permeability both in blood and lymphatic vessels, and helping cancer cells to enter the lymphatics and get established in lymph nodes, may offer a possible explanation for the observed association. This result may confirm the important role of VEGF induction for neovascularization that allows the cancerous cells to migrate to the surrounding lymph nodes.

Tae et al, (2000) and Wong et al, (2003) [10,11] recorded similar finding. While, Kyzas et al, (2004) and Shintani et al, (2005) [12,13] reported a non- significant difference in VEGF expression in OSCC with and without lymph node metastasis.

Several studies reported that no correlation was found between VEGF expression and lymph node metastasis [12,13,16,17,18], whereas, VEGF expression was reported to be related to lymph node metastasis [11,19,20].

The existence of variable VEGF isoforms, the cross-reactivity of antibodies, in addition to the different methods used to assess VEGF expression and by including tumors with different sites in head and neck region might lead to the conflicting results in different studies.

A statistically significant difference was recorded in VEGF expression in different histopathological grades with a highly significant positive correlation with histopathological grades. That means, VEGF expression correlated well with the development of oral cancer and tumor differentiation.

Shintani et al, (2005) and Johnston and Logan (2007) [13,21] reported similar finding. While other studies reported a non-significant difference and no correlation between VEGF expression and histopathological grades [10,11,12,22].

VEGF expression in tumor with inflammatory cells that stained positively for VEGF in which the macrophages were the most predominant type of inflammatory cells in these positive specimens rather than lymphocytes, was statistically significantly differed than that with inflammatory cells negative for VEGF expression and strong correlations were recorded regarding VEGF expression in tumor cells and inflammatory cells. This result indicated that both carcinoma and non-neoplastic inflammatory cells were identified as contributors of VEGF expression in the tissue implying that both cell types may stimulate angiogenic process. Monocytes are recruited into tumors from the circulation along defined chemotactic gradients, and they then differentiate into tumor associated macrophages and then accumulate in avascular and necrotic areas, where they are exposed to tumor hypoxia, since most solid tumors have focal hypoxic areas that cause low oxygen tension and regularly contribute to tumor necrosis [23,24].

Several studies reported that some of the inflammatory cells infiltrating the tumors, especially macrophages, were found to stain strongly for VEGF [10,12,20,21]. Murdoch et al, (2004) and Sica et al, (2006) [25,26] reported that tumor associated macrophages (TAMs) release a number of potent pro-angiogenic cytokines, such as VEGF, TNF-α, IL-8 and bFGF.

No statistically significant difference in MvD among different tumor sizes, in addition tumor size in T4 stage showed the highest vascularity than other tumor sizes (mean of intratumoral MvD=37.69 and mean of peritumoral MvD=43.38).Several studies reported similar results [10,11,12,13]. While Gleich, et al, (1996) [16] found a positive correlation between MVD and tumor size. This could be explained, as tumors grow, MVD increases parallel to tumor volume, as assessed by T stage. MVD is maintained during carcinogenesis and increases with tumor growth. These characteristics suggest that some

angiogenic factors released from OSCCs promote neovascularization [13].

No statistically significant difference between histopathological grades and MvD, was recorded, and the poorly differentiated tumor showed high vascularity than other histopathological grades (mean of intratumoral MvD=38.33 and mean of peritumoral MvD=43.66). This may be due to, poorly differentiated cancer cells secrete VEGF more than the well differentiated cancer cells, in order to provide enough vascularity for the cancer to grow and metastasize.

Several studies reported that no significant difference and no correlation was found between MvD and histopathological grade [10,12,19,22,27]. While Wong et al, (2003) [11] reported a significant difference between MvD and histopathological grade and he recorded that the poorly differentiated tumor showed high vascularity than other histopathological grades.

In head and neck cancer, there were conflicting data about correlation between tumor MvD and metastasis. Some studies showed association between them [28,29]. Whereas in other studies however, there was no such correlation with clinical para Meters [10,30]

A statistically significant difference in MvD whether, there is lymph node metastasis or not, was recorded, but in a weak correlation was found between MvD and lymph node metastasis. This means that lymph node metastasis does not depend on angiogenesis only, but other factors may be responsible for tumor progression and metastasis.

Several studies reported that no significant difference and no correlation were found between MvD and lymph node metastasis [10,12,13,19]. While other studies reported that a correlation was found between MvD and lymph node metastasis [11,19,22,31]. Moriyama (1997) [19] stated that some patients developed Lymph node metastasis(LNM)despite showing the lowest levels of MvD. The diverse results of various studies may be due to different tumors having varying dependencey on new vessels for neoplastic development and progression, the disparities in methodology, and the difficulty in distinguishing preexisting vessels from neovascularisation (Moriyama, 1997) [19].

In this study, a significant difference, but a weak correlation was found in MvD with the different intensities of inflammation. This could be explained as that VEGF expression by the inflammatory cells may play a role in the induction for angiogenesis side by side with the tumor cells and they are both responsible for increase MvD.

In the present study, there was a highly significant difference, in MvD with different degrees of VEGF expression (both intensity and positivity) but a weak correlation between VEGF positivity and MvD and strong correlation between VEGF intensity and MvD. That means, tumors with high expression of this inductive angiogenic factor VEGF (both positivity and intensity) tended to have high MvD.

Some studies reported a highly significant difference and strong correlation between VEGF Expression and MvD [13,32,33]. While, other studies reported no significant difference and no correlation between VEGF Expression and MvD [10,11,12,16,18,19]. Carlile et al in (2001) [34] stated that the existence of variable VEGF isoforms together with the cross-reactivity of antibodies might lead to the conflicting results in different studies.

Until now, to our knowledge no previous study mentioned the possible influence of inflammation in OSCC regarding angiogenesis, and how to deal with it when identifying the areas of the highest vascular density.

Some studies reported that a strong correlation was observed between tumor associated macrophage densities and vascular densities in many human tumor types, suggesting that tumor associated macrophages regulate neovascularization [24,31,36].

The analysis of the present study results showed that MvD increased with increasing degree of inflammation indicated by the moderate to weak correlation.

Moreover, MvD in tumors in which the inflammatory cells where positive for VEGF was significantly increased than those tumors with negative expression of inflammatory cells for VEGF, but a weak correlation was found in this context. The possible explanation of this, that the inflammatory cells, especially macrophages may play a role in tumor neovascularization, by secreting angiogenic molecules particularly VEGF. These results supported the findings of other studies [1,2,24,37] who stated that tumor angiogenesis is mediated by growth factors produced by tumor cells and/or by tumor-infiltrating inflammatory cells such as macrophages or neutrophils.

Although, only six OSCC specimens showed inflammatory cells stained positively for VEGF and the remaining were negative for VEGF immunostaining. This is suggested that most of inflammatory cells infiltrating the tumor were lymphocytes. No study until now confirmed the responsibility of these cells to produce VEGF.

Albini et al, (2005) [3] stated that inflammation- dependent angiogenesis seems to be a central force in tumor growth and expansion, and the mechanisms of inflammatory angiogenesis provide new approaches to target, cure, or even better; prevent tumor angiogenesis by treatment with synthetic or natural agents with anti-inflammatory properties.

The findings of the present study revealed that, a significant association between increasing degree of inflammation and rising estimates of angiogenesis described by increase in MvD, since VEGF stained sections demonstrated both carcinoma and host inflammatory cells (mainly macrophages) as contributors of VEGF to the tissues. Since inflammatory leukocytes might provide the angiogenic stimulus in the initial phases of tumerigenesis, as well as growth stimulus permitting accumulation of further mutations that eventually renders the tumor malignant [3].

CONCLUSIONS

In conclusion, our finding suggests that vascular endothelial growth factor is upregulated in oral squamous Cell carcinoma, which confirms the important role of this factor in induction for tumor angiogenesis, thus VEGF upregulation in OSCC, tend to be associated with increase in the MvD.

Inflammatory cells, especially macrophages may play a role in tumor neovascularization, by secreting VEGF, thus MvD was significantly increased when the tumor associated inflammatory cells where positive for VEGF.

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پوختە

دەستنیشانکرنا فاکتەرێ دەماران یێ پەنجەشێرێ و کولبوونێ (VEGF) ب گرێدان دگەل پربوونا هیردەماران ل پەنجەشێرا دەقی

پێشەکی و ئارمانج: مەزنبوونا پەنجەشێران گرێدان ب چێبوونا دەماران قە هەیە یا کو گرێدان ب هندەك فاکتەرێن مەزنبوونێ قە هەی وەکی VEGF. ئارمانجا سەرەکی یا قەکولینێ دەستنیشانکرنا هەبوونا قی فاکتەری یە ژ خانێن پەنجەشێری ب گرێدان دگەل پربوونا هیردەماران ل پەنجەشێرا دەقی.

ڕێکێن قەکولینێ: دقێ قەکولینێ دا 30 پارچێن پەنجەشێری یێن کو هاتینە دەستنیشانکرن وەك پەنجەشێرا دەقی هاتنە قەکولین کرن. ئەڤ نمونە هاتنە رەنگکرن ب ڕێکێن کیمیایی ب دژەلەشێن دژی VEGF و CD34.

ئەنجام: هەبوونا VEGF گرێدانەکا ناڤنجی هەبوو دگەل جهێ پەنجەشێرێ،بەلێ گرێدان گەلەکا توند بوو دگەل قەبارێ پەنجەشێرێ و قوناغا پێشڤەچوونا پەنجەشێرێ. هەروەسا گرێدان یا لاواز بوو دگەل گرتنا گرێکێن لمفی ب پەنجەشێرێ. پەیوەندیەکا باش هەبوو د دیارکرنا VEGF دناڤبەرا خانێن پەنجەشێرێ و یێن کولبوونێ. پربوونا هیردەماران گرێدانەکا توند هەبوو دگەل قەبارە و جهێ پەنجەشێرێ و گرێدانەکا ناڤنجی دگەل قوناغا پێشڤەچوونا پەنجەشێرێ. جیاوازیەکا گەلەك باش دیاربوو دناڤبەرا پربوونا هیردەماران دا و هەبوونا VEGF.

دەرئەنجام: VEGF یێ گرێدایە دگەل دروستبوونا دەمارێن پەنجەشێرێ. زێدەباری کو هەردوو خانێن پەنجەشێری و یێن کولبوونێ د دەستنیشانکری نە کو بەشدارن د هەبوونا VEGF دا.

الخلاصة

الكشف عن محفز تكوين الأوعية للخلايا السرطانية والالتهابية عن طريق عامل النمو VEGF بالعلاقة مع تكثف الأوعية الدقيقة في سرطان الفم

خلفية وأهداف البحث: نمو الورم الخبيث هي عملية تعتمد على تكوين الأوعية الدموية والمتعلقة بدورها بعوامل النمو مثل VEGF. ان الهدف الرئيسي للبحث هو الكشف عن اظهار العامل VEGF من الخلايا السرطانية والالتهابية وعلاقته مع تكثف الأوعية الدقيقة في سرطان الفم.

طرق البحث: شمل البحث 30 مقطع نسيجي مما شخص بأنه سرطان الفم من نوع الخلايا الحرشفية. اصطبغت هذه المقاطع بالطرق المناعية الكيميائية بمضادات ال VEGF وال CD34.

النتائج: كانت هناك علاقة متوسطة بين اظهار ال VEGF و موقع الورم، بينما كانت العلاقة قوية مع حجم الورم ودرجة تطوره. والعلاقة كانت ضعيفة مع شمول العقد اللمفاوية بالسرطان. كانت هناك علاقة متوسطة القوة بين اظهار ال VEGF من قبل كلتا الخلايا السرطانية و الالتهابية.

وجدت علاقة قوية بين تكثف الأوعية الدقيقة مع حجم الورم و موقع الورم، بينما كانت العلاقة متوسطة مع درجة تطور الورم. وكان هناك فرق معنوي كبير بين تكثف الأوعية الدقيقة واظهار ال VEGF مع علاقة ضعيفة بينهما. والعلاقة كانت ضعيفة جداً بين اظهار ال VEGF في الخلايا الالتهابية و تكثف الأوعية الدقيقة.

الاستنتاج: ان تنظيم ال VEGF متعلق ب تكوين الأوعية الجديدة في الورم. اضافة الى ذلك فان كلتا الخلايا السرطانية والالتهابية تعتبر مساهمات في اظهار ال VEGF في الأنسجة.

THE EFFECT OF CIGARETTE SMOKING ON PHARYNGEAL BACTERIAL FLORA AMONG STUDENTS OF DUHOK COLLEGE OF SCIENCE

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ABSTRACT

Background: Cigarette smoking is associated with acute respiratory tract infections in adults. Active smoking and passive exposure to cigarette smoke is also associated with carriage of some potentially pathogenic bacteria. The direct effects of smoke on the growth of many bacterial species in upper respiratory tract are mostly unknown, although the effect of smoke on dental flora has been examined extensively.
Objectives: To compare the isolation rate of pharyngeal bacterial normal flora and potential pathogens between non-smokers (30) and smokers (30) among students in College of Science in Duhok University and to compare the number of colonies (density of growth) of each bacteria among smokers and non-smokers.
Methods: Cotton swabs were used to rub the tonsils and areas adjacent to the tonsils and inoculated into media plates: blood, chocolate and MacConkey media plates. Bacteria were identified according to their cultural morphology, gram staining and biochemical tests. The number of each isolated bacteria and the extent of their growth on plates were compared among both the smokers and the non-smokers.
Results: Eight types of bacteria were identified from both smokers and non-smokers. The rate of *Viridance streptococci, Neisseria spp,* and *Diphtheria spp*, were similar for both smokers and non-smokers but the rate of *Bacillus spp*.were significantly less among smokers performed by chi-square test (p: 0.045) compared to non-smokers. There was also a significant decrease in the extent of growth of *non-coagulase staphylococci* among smokers compared to non-smokers calculated by Mann-Whitney test (p: 0.044). There were four episodes of *Staph. aureus* among the smokers but non among non-smokers.
Conclusion: the decrease in both the growth and the extent of growth in some of bacterial normal flora among smokers such as *Bacillus ssp* and *non-coagulase staphylococci* respectively might help potential pathogens such a *Staphylococcus aureus* to colonize the epithelial flora. These potential pathogens might play an aetiological role in respiratory tract infection.

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Key words: Cigarette smoking, Pharynx, Bacterial flora, Duhok.

Smoking is associated with an increased risk of respiratory tract infection in adults1 and also with oral colonization by some potentially pathogenic microorganisms [2,3]. Micro-flora generally consists of saprophytic microbes which are acquired during and after few days of birth of an individual. Bacteria are predominant normal flora organisms. They have an extraordinary ability to attach, colonize epithelial cells and establish in human body. Human being has specific normal flora and its composition is dependent on health status, diet, age and hormonal activities of that individual. Reduction in populations of micro-flora can promote opportunistic infections in body. Opportunistic pathogens are actually resident microbes which takes an opportunity to induce infection. They also cause infection when they get entry into another region of the body.

In children, exposure to cigarette smoke is a risk factor for respiratory tract infection and meningococcal meningitis [4]. Active smoking and passive exposure to cigarette smoke is also associated with carriage of potentially pathogenic species of bacteria in both adults and children [5] possibly owing to enhanced bacterial binding to epithelial cells of smokers [6].

The nasopharyngeal flora of smokers

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harbour fewer aerobic and anaerobic organisms with interfering capability and more potential pathogens compared with those of non-smokers [7]. It is important to determine these direct effects of cigarette smoke on bacterial species and determine the contribution of this reselection of the species in the pathogenesis of bacterial infection in smokers.

METHODS

Thirty healthy male adult students (age from 18- 30 years) of Biology Department/College of Science/University of Duhok who smoke at least 10 cigarettes or more per day and thirty non-smokers with the same range were included in the study. None of them had a respiratory tract infection at least 3 months prior to the time when samples were taken.

The oropharynx culture specimens were sampled using nylon-flocked swabs (Copan). The posterior oropharynx was sampled trans-orally adjacent to the tonsillar pillars. After collection, swabs were immediately plated into three media plates: Blood agar, chocolate, and Mac-Conkey (prepared in our lab). Blood and chocolate plates were incubated in CO2 incubator at 37°C while Mac-Conkey plates were incubated in an incubator without CO2.

The plates were examined after 24-48 hours of incubation and all colonies on each plate were isolated. Aerobic bacterial isolates from each individual 8 sub-cultured and further identified.

Identification of bacterial isolates was performed depending on morphological cultural characteristics, Gram stain, and biochemical tests: oxidase, catalase, and manitol salt agar9. Oxidase test is positive for *Bacillus spp* and *Neisseria spp*. Manitol salt agar was used to differentiate between staphylococcus aures which gave yellow colour and *non-coagulase staphylococci* which did not show yellow colour.

For density of bacteria, 3 sector streak technique was performed. The number and extent of growth of each isolate was determined according to the extent of growth in relation to the sector of streaking. Low growth was decided if the colonies were grown only on the first streak, moderate growth was grown on the first and second streaks and heavy growth was extended to the third (last) streak.

RESULTS

Eight different types of the normal bacterial flora were isolated from both the smokers and the non- smokers. (Table 1) shows the rate of different types of bacterial flora among both smokers and non-smokers.

Table1. Number and rates of bacteria isolated from smokers and non-smokers.

Types of bacteria	Non-smokers (30)	Smokers (30)
Viridance streptococcus	**28**	**29**
Neisseria spp	**29**	**29**
Bacillus spp	**12**	**5**
Diphtheria spp	**1**	**2**
Non-coagulase spp	**18**	**11**
Staphylococcus aureus	**0**	**4**
Gram Negatice bacilli (Non-fermenters)	**1**	**2**
Candida spp	**2**	**0**

Statistical analysis was performed using chi-square revealed that the rate of *Bacillus spp*. was significantly less among smokers compared to non-smokers p: 0.045. The rates of the other isolated bacteria were not significantly different between both groups.

Results for the extent of growth (number of colonies in media plates) were determined for all the isolates in both non-smokers and smokers (Tables 2 and 3).

Table 2. The extent of bacterial growth among non-smokers.

Type of bacteria in non-smokers	Low growth	Moderate growth	Heavy growth
Viridance streptococci (28)	1	8	19
Neissreiaspp (29)	4	9	16
Bacillus spp (12)	5	5	2
Non-coagulase staphylococci (18)	4	11	3

Table 3. The extent of bacterial growth among smokers.

Type of bacteria among smokers	Low growth	Moderate growth	Heavy growth
Viridance streptococci (28)	3	10	15
Neissreia spp. (29)	6	18	5
Bacillus spp. (5)	1	3	1
Non-coagulase staph (11)	8	2	1

Statistical analysis was performed using Mann-Whitney test for comparison revealed that the extent of growth for *Non-coagulase staphylococcus* is significantly lower in smokers compared to non-smokers (Table 4). Results for other types of bacteria were not significant.

Table 4. P values for extent of bacterial growth of smokers versus non-smokers.

Bacteria	P values by Mann-Whitney Test
Viridance Streptococci	P= 0.827 not significant
Neisseria spp	P= 0.827 not significant
Bacillus spp	P= 0.116 not significant
Non-coagulase staphylococcus	P= 0.044 significant

DISCUSSION

This study focused on the spread of aerobic bacterial normal flora and potential pathogens of Upper Respiratory Tract lnfections in smokers and non-smokers among adult students in Duhok city. Almost every smoker and non-smoker carried *Viridance streptococci* and *Neissreia spp* on their pharynx. Among the non-smokers 12 students carried *Bacillus spp* while only 5 smokers carried *Bacillus spp*. and this decrease was statistically significant when performed by chi-square test (p: 0.045). A similar trend is observed for *non-coagulase staphylococcus* as 18 of the non-smokers were carriers but only 11 of the smokers were carriers however this decrease was not significant.

Staphylococcus aureus is regarded as potential pathogen in upper respiratory tract which causes lung infection (pneumonia) in some individuals. *Staphylococcus aureus* was not isolated from non-smokers but was detected in 4 of the smokers. Other studies have shown similar results [10] The rate of potential pathogenic bacteria may be more evident in a larger scale study considering other factors.

Smoking increases the acquisition of periodontal pathogens and periodontal disease, colonization by respiratory pathogens, and the occurrence of upper respiratory tract infection. The reduction in the number of members of the normal flora that interfere with the growth of pathogens and the greater adherence of bacterial pathogens to the oral mucosa are associated with the greater frequency of respiratory infections including otitis media. The flora of smokers contains fewer aerobic organisms with interfering activity against bacterial pathogens and harbours more potential pathogens as compared with the flora of non-smokers [11,12].

Regarding the number of colonies (density of growth) there was a significant decrease in the number colonies of *non-coagulase staphylococci* among smokers compared to non-smokers when calculated by Mann-Whitney test (p: 0.044). Also, there is a similar trend for *Neisseria spp*. but it was not significant. The decrease in density of some of bacterial normal flora among

smokers such as *Neisseria spp*, *non-coagulase staph* and *Bacillus spp* might help potential pathogens to colonize the epithelial flora such as *Staphylococcus aureus* and gram negative bacilli. These potential pathogens might play a role in lung infection.

Analysis of the data presented illustrates the adverse effects of direct exposure to smoking on normal bacterial flora and colonization with potential pathogens. The normal flora prevents colonization by pathogens by competing for attachment sites. This is thought to be their most important beneficial effect, which has been demonstrated in the oral cavity, the intestine, the skin, and the vaginal epithelium [13].

Further studies on greater number of smokers are helpful to investigate whether colonization of the nasopharynx with potential organisms and/or the cessation of smoking would allow for the return of the normal bacterial flora and a reduction in the number of pathogens.

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پوختە

کاریگە ریا جگارە کێشانێ لسە ر بە کتیریایێن جێگیر دناف گە وریا قوتابیێن فاکولتیا زانست لـ زانکویا دهوك.

ناڤەروك و ئارمانجێت ڤەکولینێ :جگارەکێشان دبیتە ئەگەرێ توشبوون بـ٘نەخوشیێت هەناسێ بێن گران ل دەف کەسانێن پێ گەهشتی (Adult) و دبیتە ئەگەرێ زێدەبونا ژمارەکا زور یا بەکتیریا بێن کو دبنە ئەگەرێ نەخوشیا ل دەف کەسانێن جگارە کێش.کارتێکرنا جگارەکێشانێ کو دبیتە ئەگەرێ گەشەپێدانا ژمارەکا زور یا بەکتیریا ل ئەندامێ هەناسەکرنێ یێ سەری ,هەتا نوکە نەیا ئاشکرایە لێ پا کارتێکرنا جگارەکێشانێ ل سەر بەکتیریا ئەوێن بشێوەیەکێ سروشتی دناڤ دەڤی دا هەین بەری نوکە د ڤەکولینا دا هاتیە کرن.

ئارمانجێت ڤەکولینێ: بەراورد کرن دناڤ بەکتیریا بێن سروشتی و بێن نەخوشی دناڤ گەوریا ئەو قوتابیێن جگارا نەکێشن (30 قوتابی) و قوتابیێن جگارە کێش (30 قوتابی) ل فەکەلتیا زانست ل زانکویا دهوك) , هەروەسا بەراوردکرن د ناڤبەرا داگیرگەرێن بەکتیری بێن سروشتی و نەخوشی هاتە کرن.

شێوازێ کارکرنا ڤەکولینێ: ب رێکا زەوی سرێ لوکەی (Cotton swab) نموونەك ژ بەکتیریا هاتە برن ژ ل بەلاعینا و دەوروبەرێت وان و پشتی ڤێ چەندێ گەشەبێ هاتە کرن لسەر خارنێن گەشەپێدانا بەکتیری هەر ئێك ژ (Blood agar, MacConky agar and Chocolate agar) و لدویف دا جورێن بەکتیریا هاتە هژمارتن ب رێکا شێوێ داگیرگەرا , و بویاغا گرام (Gram stain) و پشکنیێن کیمیاوی بو هاتنە کرن. هەمی ئەوان جورێن بەکتیریا ئەوین هاتین دەست نیشان کرن هاتنە بەراوردکرن د ناڤبەرا کەسێن جگارەکێش و جگارە نەکێش دا.

ئەنجام: هەشت جورێت بەکتیریا هاتنە دەست نیشان کرن ل دەف کەسێن جگارەگێش و جگارەنەکێش دا و رێژا ئەنجامێن بەرواوردکرنێ د ناڤبەرا هەر ئێك ژ بەکتیریایێن (*Diphtheria, Neisseria, Viridance streptococci*) یا یەکسان بوو.

رێژا بەکتیریا Bacillus ل دەف کەسێن جگارەکێش کێمتربوو ب بەراوردکرن دگەك کەسێن جگارەنەکێش و ب پێ ژمارە ئاماری (P = 0.044) کو ب رێگا تاقیکرنا Chi-Square هاتبوو کرن ,هەروەسا رێژا گەشەپێدانا (*Non-coagulate Staph*) ل دەف کەسێن جگارەکێش زور یا کیمتربوو ب بەراوردکرن دگەك کەسێن جگارەنەکێش (P = 0.044) ب پێ تاقیکرنا Mann-Whitney , هەروەسا 4 کەسا بێن جگارەکێش گەشەپێدانا بەکتیریا *Staphylococcus aureus* هاتە دیتن ب بەراوردکرن دگەك کەسێن جگارەنەکێش .

دەرئەنجام: کێم بوون ب گەشەپێدانا بەکتیریا *Bacillus* و هەروەسا کێم بوون ب گەشەپێدانی چریا *Non-coagulate Staph* هاتە دیتن ل دەف کەسێن جگارەکێش ب بەراوردکرن دگەك کەسێن جگارەنەکێش کو ئەڤەژی دبیتە پالدەرەك بو گەشەپێدانا بەکتیریا نەخوشی *Staphylococcus aureus* لسەر بەلاعینا و دەوروبەریت وان کو دبیتە ئەگەر ب توشبونا هەلگراندنا ئامیرێ هەناسێ ل دەف کەسێن جگارەکێش.

الخلاصة

تأثير التدخين على البكتريا المستوطنة في البلعوم لدى طلبة فاكولتي العلوم في جامعة دهوك.

خلفية واهداف البحث:التدخين يصاحبها امراض تنفسية حادة لدى الكبار وكذلك يحمل المدخنين ومرافقي المدخنين عدد من البكتيريا المرضية.التأثير المباشر للتدخين على نمو بعض البكتيريا في الجهاز التنفسي العلوي غير معلوم ولكن تاثير التدخين على البكتيريا الطبيعية المتواجدة في الفم قد تم دراستها سابقا.

أهداف البحث: مقارنة أنتشار البكتيريا الطبيعية و المرضية في البلعوم لدى الطلبة غير المدخنين و المدخنين (30)في كلية العلوم بجامعة دهوك وكذلك مقارنة اعداد المستعمرات (كثافة النمو) لهذه البكتيريا لدى المدخنين وغير المدخنين.

طرق البحث: تم أخذ مسحات من اللوزتين والمناطق المحيطة باللوزتين في البلعوم وتم زراعتها على أوساط: Blood, Chocolate, and MacConkey تم التعرف على البكتيريا حسب أشكال المستعمرات, صبغة كرام و من الفحوصات الكيميائية. تم مقارنة كل نوع من البكتيريا وكثافة النمو بين المدخنين وغير المدخنين.

النتائج: تم التعرف على ثمانية أنواع من البكتيريا لدى كل من المدخنين وغير المدخنين وكانت نتائج المقارنة نسب كل من *Viridance Streptococci, Neisseria, Diphtheria* لدى المدخنين وغير المدخنين متساوية.

كانت نسب *Bacillius* اقل لدى المدخنينمقارنة بغير المدخنين وأحصائيا كانت النسبة(P=0.045) حسب فحصChi-Sequare وكذلك كانت كمية نمو *Non-coagulase Staph* لدى المدخنين اقل بكثير مقارنة بغير المدخنين (P=0.044) حسب فحص Mann-Whitney, كذلك كانت هناك 4 حالات لنمو*Staphylococcus aureus* لدى المدخنين ولكن ليس لدى غيرالمدخنين.

الخلاصة: النقصان في نمو ال*Bacillius* وقلة كثافة النمو ل*Non-coagulase Staph* عند المدخنين مقارنة لغير المدخنين سيساعد البكتيريا المرضية مثلا *Staphylococcus aureus* للنمو في هذه المناطق حيث لها دور في التهابات الجهاز التنفسي.

GROWTH STATUS OF A SAMPLE OF CHILDREN WITH TYPE 1 DIABETES MELLITUS IN BAGHDAD GOVERNORATE

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ABSTRACT

Objective: to assess the growth status of a sample of children age (2 to 5) years with type 1 diabetes mellitus by using weight, height , head, mid upper arm circumferences.
Methodology: A descriptive, cross- sectional study was done in diabetic clinics and hospitals from each side of Baghdad from the period 9th of November 2009 to the 1st of March 2010, data collected by direct interview with child's parents using especially designed questionnaire and review of medical records.
Results: The results showed that the mean weight and height was higher in males than in females for all age groups with significant at age (3), (5), (7) years and it was close to the international standards. The mean head circumference was higher in males than in females for all age groups with significant at age (3), (4,5) years and it was under normal limits of the international standards. The results showed that (17.9%) of children had wasting , and the frequency of patients within underweight was (7.3%) , about (6.8%) of the sample were stunting , and about (5.7%) were thin.
Conclusions : It was found that in all measurements males had higher mean values than females and both showed normal growth distribution. The present study showed that the majority of children under 5 years have normal weight for height index and a minority of them have wasting, small proportion of under-weight occurred in children under 10 years. Highest percentage of children have normal height for age and lowest percentage of them suffering from stunting. The majority of children have normal BMI for age and minority of them were thin. Socio-economic status, age, and duration of the disease is significantly associated with height for age index.

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Key words: Growth, Type 1 diabetic

Type 1 Diabetes Mellitus(DM) is the most important metabolic disease in children and adolescents; which may lead to delayed growth and puberty [1]. Several decades ago, insulin dependent diabetes mellitus used to be listed among causes of severe growth retardation in children [2]. Epidemiological findings from the united kingdom have led to the hypothesis that under-nutrition in utero and during infancy probably changes the body's physiology and metabolism [3]. A severe metabolic disease like type 1 DM is likely to influence the growth profoundly. Children with type 1 DM, with the onset of their in the pre pubertal period are concerned about their growth particularly reduction in height.[4,5] Moreover weight loss is a cardinal feature of childhood diabetes. This situation is rapidly reversed after the initiation of insulin therapy [6]. Growth and development of children with diabetes depend on the adequacy of insulin administration and degree of metabolic control. Some studies showed growth retardation in diabetic children can occure regardless of the degree of metabolic control [7]. Children who are appropriate on doses of insulin and, as a result , have well-controlled diabetes, typically have patterns of growth and development that are identical to those of otherwise healthy children[8].

METHODS

A cross sectional study was conducted during the period between 9th of

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November 2009 to the 1st of March 2010. A convenient sample of 550 patients i.e all available cases at that period has been collected from different sites including: The National Center for Treatment of Diabetes of AL-Mustanseriya University, The Specialized Center for Endocrinology and Diabetes (situated at ALkindy hospital), Child Central Teaching Hospital, The Children Welfare Hospital, and Ibin El- Baladi Pediatric and Maternal Teaching Hospital.

Data collection: by direct interview with child's parents using a special-designed questionnaire that included the pertinent information and review of medical records.

Instruments used:

Weight was measured while the child standing without shoes using the same scale for all children (Unicef uniscale).

Height measured at the time of interview while the child is standing without shoes using board with a horizontal head that can be brought in contact with the upper most point on the head.

Head circumference: was measured by using a narrow (less than 1 cm wide) flexible, non – stretch tape made of fiberglass. The greatest circumference of the head was measured by placing the tape firmly round the frontal bones.

Statistical methods: the data was analysed by using SPSS v 14 and the results were tested by using t test for association between two variables with results being considered as statistically significant when the p value was < 0.05.

RESULTS

(Table 1) shows that the mean weight for age in males was higher than in females except in age (9 years) and the difference was significant in ages (3), (5), (7), (9) years.

Table 1. Distribution of mean weight (Kg) according to age in both genders

Age years	No. Males=276	No. Females=274	Males Mean	Males SD	Females Mean	Females SD	P- value of t-test
2	10	7	11.82	0.97	10.99	1.38	0.161
3	20	13	14.49	1.51	13.48	0.106	0.040
4	28	17	16.05	2.13	15.08	2.08	0.139
5	15	14	17.90	2.49	16.40	2.12	0.049
6	20	25	19.09	1.84	18.74	1.67	0.508
7	29	28	23.32	2.51	20.96	2.95	0.002
8	16	29	24.83	3.21	24.44	3.17	0.698
9	44	27	27.88	3.20	34.54	6.17	0.461
10	35	28	30.79	3.99	29.95	4.05	0.408
11-12	59	86	35.39	4.49	34.66	4.15	0.315

(Table 2) represents that the mean Height for age was higher in males than females in all ages and the difference was significant in age (2), (3), (5) and (7) years,

Table (3) shows that the mean head circumference was higher among males than females in all ages and the difference was significant in age [(3),] (4-5) years.

(Figure 1) illustrates the growth curve in males. The graph shows steady increasing in mid upper arm circumference (MUAC) after the end of 5th years. While (Figure 2) in females shows almost increasing in MUAC after the age of 5 years.

Table 2.Distribution of the mean Height (cm) according to age .

Age years	No.		Males		Females		P- value of
	Males=276	Females=274	Mean	SD	Mean	SD	t-test
2	10	7	84.630	2.462	83.157	4.835	0.479
3	20	13	94.330	3.835	91.985	3.258	0.047
4	28	17	100.696	4.803	98.206	4.639	0.095
5	15	14	108.760	5.694	105.029	4.886	0.042
6	20	25	112.200	5.948	112.024	4.564	0.911
7	29	28	120.383	3.855	117.968	5.518	0.039
8	16	29	124.544	6.802	123.617	4.976	0.603
9	44	27	129.968	5.042	128.004	5.131	0.118
10	35	28	132.900	5.537	132.346	5.590	0.696
11-12	59	86	140.764	5.743	139.9035	4.935	0.336

Table 3. Distribution of the mean head circumference (cm) according to age.

Age Years	No.		Males		Females		P- value of
	Males	Females	Mean	SD	Mean	SD	t-test
2	10	7	46.42	1.77	45.21	2.02	0.212
3	20	13	47.62	1.85	46.35	1.30	0.040
4-5	28	17	47.87	1.69	45.85	1.21	0.00

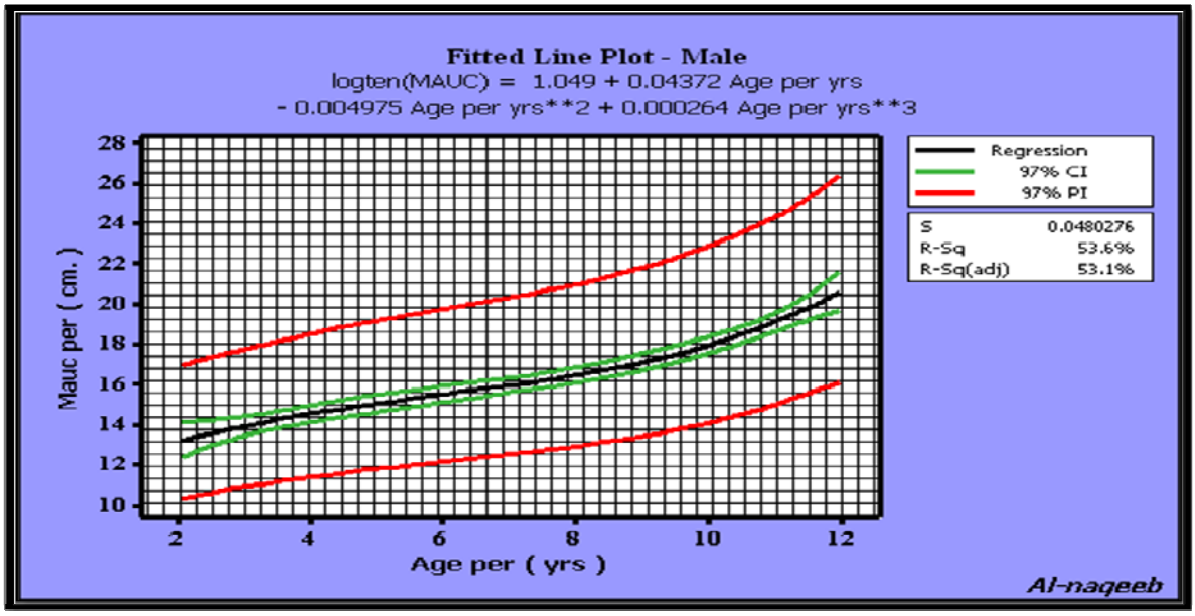

Figure 1. Fitted line of 97% predicted MUAC for males (2-12) years age.

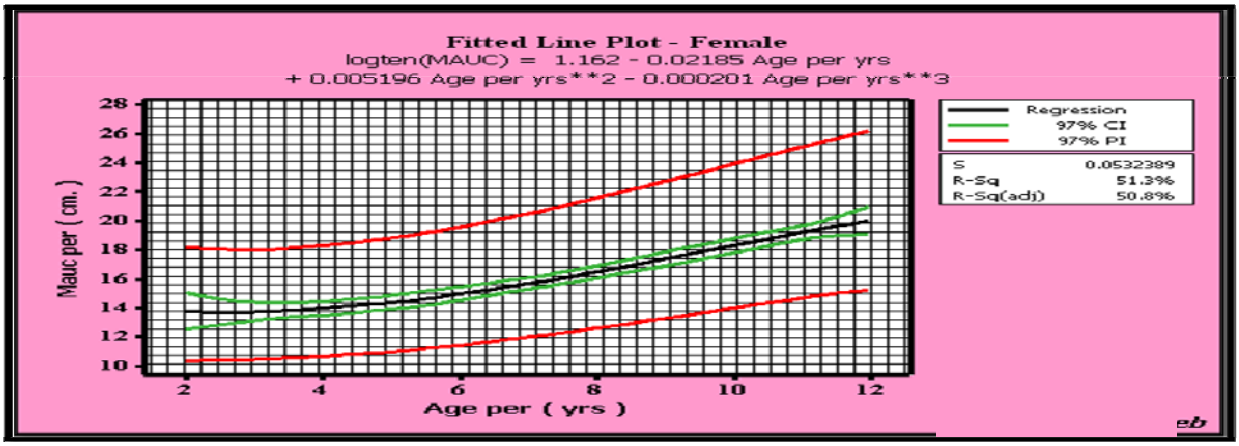

Figure 2. Fitted line of 97% predicted MUAC for females of (2-12) years.

(Table 4) table reveals that (17.9%) of children less than 5 years had wasting, and the proportion of patients within normal was (76.8%). under weight includes (7.3%) of children aged less than10 years, and the proportion of patients within normal was (90.1%), finally (93.2%) of the total sample were within normal height for age and the proportion of patient with normal BMI for Age was (89.2%).

Table 4. Distribution of diabetic children according to Weight for Height, weight for Age, height for Age, and BMI for Age.

Variables	No.	%
Weight for Height (N=95)		
Wasting	**17**	**17.9**
Within normal	**73**	**76.8**
Risk of over weight	**3**	**3.2**
Over weight.	**2**	**2.1**
Total *	**95**	**100.0**
Weight for age (N=342)		
under weight	**25**	**7.3**
Within normal	**308**	**90.1**
Risk of over weight	**8**	**2.3**
Over weight.	**1**	**0.3**
Total **	**342**	**100.0**
Height for age (N=550)		
Stunting	**37**	**6.8**
Normal	**513**	**93.2**
Total ***	**550**	**100.0**
BMI for age (N=550)		
Thin	**31**	**5.7**
Within normal	**491**	**89.2**
Risk of over weight	**26**	**4.7**
Over weight	**2**	**0.4**
Total ***	**550**	**100.0**

** It includes children from birth to 5 years*
*** It includes children from birth – 10 years*
**** It includes all the sample.*

(Table 5) reveals that stunting occurred more frequently in the age (2 years). The proportion of stunting in females (8%) was higher than that among males (4.7%) and all children with high socio- economic status had normal height for Age z score (99.0%) compared to (91.3%) of diabetic children with low Socio- economic status.

Table 5 . Distribution of the studied sample according to their Height for Age z score categories and some socio-demographic variables.

Variables	Sever stunting N	%	stunting N	%	Within Normal N	%	Total N	%	ANOVA P-value
Age in (yrs.)									
2	**0**	**0**	**7**	**41.2**	**10**	**58.8**	**17**	**100**	**0.00(HS)**
3	**1**	**3.0**	**2**	**6.1**	**30**	**90.9**	**33**	**100**	
4	**0**	**0**	**1**	**2.2**	**44**	**97.8**	**45**	**100**	
5	**0**	**0**	**4**	**13.8**	**25**	**86.2**	**29**	**100**	
6	**1**	**2.2**	**7**	**15.6**	**37**	**82.2**	**45**	**100**	
7	**0**	**0**	**4**	**7.0**	**53**	**93.0**	**57**	**100**	
8	**0**	**0**	**1**	**2.2**	**44**	**97.8**	**45**	**100**	
9	**0**	**0**	**2**	**2.8**	**69**	**97.2**	**71**	**100**	
10	**0**	**0**	**4**	**6.3**	**59**	**93.7**	**63**	**100**	
11-12	**0**	**0**	**3**	**2.1**	**142**	**97.9**	**145**	**100**	
Gender									
Males	**2**	**0.7**	**13**	**4.7**	**261**	**94.6**	**276**	**100**	**2.4(NS)**
Females	**0**	**0**	**22**	**8.0**	**252**	**92.0**	**274**	**100**	
Socioeconomic status									
Low	**0**	**0**	**19**	**8.7**	**199**	**91.3**	**218**	**100**	**0.04(S)**
Middle	**2**	**0.8**	**15**	**6.5**	**215**	**92.7**	**232**	**100**	
High	**0**	**0**	**1**	**1.0**	**99**	**99.0**	**100**	**100**	
Duration of disease(yrs)									
< 1	**2**	**1.0**	**14**	**6.7**	**191**	**92.3**	**207**	**100**	**0.043S)**
1-2	**0**	**0**	**6**	**5.5**	**104**	**94.5**	**110**	**100**	
3-4	**0**	**0**	**14**	**7.7**	**169**	**92.3**	**183**	**100**	
5 &more	**0**	**0**	**1**	**2.0**	**49**	**98.0**	**50**	**100**	
History of breast feeding									
Yes	**1**	**0.3**	**29**	**7.5**	**359**	**92.2**	**389**	**100**	**0.7(NS)**
No	**1**	**0.6**	**6**	**3.7**	**154**	**95.7**	**161**	**100**	

DISCUSSION

Males had mean value of weight higher than that of females at all age groups. This, finding is comparable to the finding reported by Alemzadeh et al 1995 [9] who demonstrated that weight gain and growth rate do not seem to be significantly affected by glycemic control and confirmed that linear growth velocity is dependant on weight gain and the level of growth producing hormones such as insulin like growth factor (IGF-1) are important regulators of linear growth.

Height measurements are important because low height for age can identify past under nutrition or chronic malnutrition. By comparing the results of the present study with the WHO growth charts [10], it was found that both genders had mean values of height similar to those standards. Head circumference data are needed by health care professional because this measurement is important in the

assessment of growth and in screening for abnormal head size, which is related to the brain size, and neurological status of infant and young children[11] The current study indicated that both genders had mean values lower than WHO standards. [(10)] This may be due to the inability to maintain good metabolic control in diabetic children[12] . The result of this study shows that the mean of MUAC was higher in males than in females , expect in the ages (5), (9), (11-12) years, the mean was higher in females this due to sex differences. This is similar to what was found in other studies done by Bhaskara etal 2003 [13,] and the study that done in Bangladesh 2000 [14.] Wasting comprises (17.9%) of children in this study, and the proportion of patients within under weight was (7.3%). It is in agreement with a study conducted by Ali(2002)in Iraq [12] . Who found lower frequency of patients are within wasting and underweight, due to the ability of diabetic children to maintain good metabolic control during their growth period. It was found that (6.8%) of the sample were within stunting and the proportion of thin patients was (5.7%). This study is in agreement with Ali 2002 in Iraq [12] . The results of this study indicated that there were significant differences in height for age with respect of age, socio-economic status and duration of disease. This finding is comparable to the finding reported by Lakshmi et al (2003) [15] where there was a statistically significant association between height for age and age, socio-economic status, also Hameida J (2002)[16] who found statistically significant association between height with age of the child and age.

CONCLUSIONS

Weight and height measurements for males and females showed normal distribution according to international standards. Head circumference was under normal limits of the world standard in both genders and for all age groups. Finally MUAC for age (2-5yrs) was similar or below normal limits of the world standard in both genders while, for age (5-12yrs) there is normal growth pattern.

RECOMMENDATIONS

Further follow up studies concentrating on the relationship between diabetes control and growth status are recommended to investigate this important issue among children with type 1 DM.

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پوخته

رەوشا گەشەکرنێ یا زاروکێت نەخوشیا شەکرێ جورێ ئێکێ لپارێزگەها بەغدا

ئارمانج: ئارمانجا ڤێ ڤەکولینێ هەلسەنگاندنا گەشەکرنا زاروکابوو بێت کو تووشی نەخوشیا شەکرێ بووین ژ جورێ ئێکێ، بکارئینانا پیڤەرێت سەرەکی (کێش ودرێژی وپیڤانا کەمەرا کلوخی ودەستا، دناڤ بەرا ژیێ (2 – 5) سالیادا.

شێواز: ڤەکولینەکا برگەی یە یا کو هاتیە ئەنجامدان لهندەك سەنتەرێت نەخوشیا شەکرێ ولخەستەخانا لرەخێت پارێزگەها بەغدا ژ ماوێ ناڤ بەرا چریا دووێ 2009 وتاکو ئادارا 2010 وپێزانین هاتنە کومکرن لچاڤپێکەتنێت راستەوخو لگەل دەیك وبابێت زاروکا هاتنە سازکرن، فومرێت تایبەت بێت پرسیارا وپێداچوونێت پێتڤی هاتنە تەرخان کرن.

ئەنجام: ئەنجام دیاربوون کو ناڤنجیێ کێش ودرێژیێ لتوخمێ نێر بلندترە ژ توخمێ مێ بو هەمی گروپێت ژیێ زاروکا دڤەکولینێ دا وجیاوازی دیاربوو لژێت (3) (5) (7) سالیادا وناڤنجیێ وان یێ نێزیکی ئاستێت جیهانی بوو. ناڤنجیێ کەمەرا کلوخی بلندتربوو لدەڤ توخمێ نێر لهەمی گروپێت تەمەنێ زاروکادا وجیاوازی یا مەعنەوی لتەمەنێ (3) ، (4 – 5) سالی دا وناڤنجی لژێر سنوور وتوخیبێ نورمال دابوو، وەکی ئاستێت جیهانی. هاتە تێبینی کرن د ئەنجامدا کو نێزیکی (17,9 ٪) ژ زاروکا تووشی لاوازیەکا دژوار بوون، وئەوێت تووشی نزم بوونا کێشێ بووین (7,3 ٪) ونێزیکی (6,8 ٪) یێ ژ ڤان زاروکێت هاتینە د ڤەکولینێ دا رەویلە ودقوت بوون (راوستانا وەرارێ وگەشێ) ونێزیکی (5,7 ٪) یێ ژیك د زەعیف وزاراڤ بوون ژ کێماسیا بەزی.

دەرئەنجام: شروڤەکرنا پێزانینا دیاربوو کو ناڤنجیێ توخمێ نێر بلندتر بوو ژ توخمێ مێ وهەر وەسا هاتە تێبینی کرن کو دابەشکرنا گەشێ یا سروشتی بوو. هەر وەسا دیار بوو کو پرانیا زاروکا بێت دبن ژیێ پێنج سالی دا کو پیڤەرێ کێشێ بوو درێژیێ دناڤ توخیبێ نورمال دا بوو ورێژەکا کێم ژ زاروکا تووشی رەویلە وقوت بوونێ بوون، ڤەکولینێ خویا کر کو پیڤەرێ کێشێ بو تەمەنی د سنووریّ نورمال وسروشتی دا بوو لبارا پتری زاروکا و رێژەکا کێم یا تووشی لاوازی وزەعیفیێ بووی ...

پێکڤە گرێدانەکا مەعنەوی دیاربوو دناڤبەرا پیڤەرێ درێژیێ بو تەمەنی دبارێ کومەلایەتی وئابووری دا، وژیێ زاروکی وماوێ نەخوشیێ دا.

الخلاصة

حالة النمو لعينة من ألأطفال المصابين بداء السكر من النوع ألأول في محافظة بغداد

الهدف من الدراسة: تهدف الدراسة إلى تقييم النمو عند الأطفال المصابين بداء السكري من النوع الأول باستخدام قياسات كل من الوزن والطول وقياس محيط الرأس ومحيط الذراع للأطفال من عمر (2–5 سنوات).

طريقة البحث: وهي دراسة مقطعية أجريت في بعض مراكز السكري والمستشفيات من جانبي بغداد للفترة ما بين التاسع من تشرين الثاني 2009 ولغاية الأول من آذار 2010 وتم جمع المعلومات من خلال المقابلات المباشرة مع ذوي الأطفال وضمن استمارة خاصة للأسئلة مع مراجعة السجلات الطبية.

النتائج: أظهرت النتائج أن متوسط الوزن والطول في الذكور أعلى من الإناث لكل الفئات العمرية و كانت الفروقات معنوية في الفئة العمرية (3) , (5), (7) سنوات وكان المتوسط قريب من المستويات العالمية . وكان متوسط محيط الرأس أعلى في الذكور أيضا من الإناث في كل الفئات العمرية وكان الاختلاف معنوي في الفئات العمرية (3) , (4–5) سنوات وكان المتوسط تحت الحدود الطبيعية , طبقا للمستويات الدولية. ولوحظ أيضا في النتائج حوالي (17.9٪) من الأطفال كانوا يعانون من الهزال , والذين يعانون من نقص الوزن كانت (7.3٪) , وحوالي (6.8٪) من العينة كانوا يعانون من التقزم، وحوالي (5.7٪) من قلة التشحم.

الاستنتاج : تحليل البيانات اظهر أن القييم المتوسطة للذكور أعلى من الإناث ولوحظ أيضا إن توزيع النمو كان طبيعيا. ووجدت الدراسة إن في أغلبية الأطفال تحت سن الخمس سنوات كان مؤشر الوزن للطول ضمن الحدود الطبيعية, ونسبة قليلة من الأطفال دون سن العشر سنوات يعانون من نقص الوزن, ونسبة كبيرة من الأطفال كان مؤشر الطول للعمر, لديهم ضمن الحدود الطبيعية ونسبة قليلة منهم يعانون من التقزم, كذلك أظهرت الدراسة إن مؤشر كتلة الجسم للعمر كان ضمن الحدود الطبيعية في أغلبية الأطفال ونسبة قليلة منهم يعانون من قلة التشحم. و أيضا أظهرت ارتباط معنوي بين مؤشر الطول للعمر مع الحالة الاجتماعية والاقتصادية، والعمر، وفترة المرض.

Current zinc status among a healthy population in Duhok city, Iraq

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ABSTRACT

Background and Objectives: Mild-moderate zinc deficiency is common in several developing countries, because the commonly consumed staple foods have low zinc contents and are rich in phytates, which is known to decrease the availability of zinc. Hence, the present study was conducted to determine the current zinc status among a sample selected from a healthy population in Duhok City, Iraq.
Methods: This study was conducted at the Department of Clinical Biochemistry/School of Medicine/Faculty of Medical Science-University of Duhok during the period of September 2009 to June 2010.A total of 332 healthy subjects (aged from 6 years to more than 65 years) living in different areas of Duhok city were enrolled in the study. We used a pre-tested questionnaire, which was designed to obtain information on gender, birth dates, height, weight, residence, and social status. Serum zinc level was measured for each subject. We considered subject with serum zinc concentration of <50 ug/dl zinc deficient and 50-70 ug/dl mild-moderate zinc deficient.
Results: Significantly lower mean values of serum Zinc concentrations was demonstrated in children and adolescents compared to the other age groups ($P<0.05$ and $p<0.01$) respectively. Of the three hundred thirty-two subjects, 10% (n=33) had mild-moderate zinc deficiency. Children and adolescents had a higher prevalence of zinc deficiency than adults (47.6% and53.8% Vs 3.5%). Severe zinc deficiency was not found in any of the subjects. . Significantly lower mean serum zinc concentration values were observed in subjects with lower social status and in those living in rural areas. . There was no significant gender difference in mean serum zinc concentration for all studied subjects. Subjects with normal weight had lower mean serum zinc concentration as compared to those with over weight or obese, but the difference was not significant ($p=0.09$).
Conclusions: Mild-moderate zinc deficiencies were observed in the studied subjects with a higher prevalence in children and adolescents, and thus it necessitates the need for an effective public health intervention means to improve the zinc status of the population

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Key words: Zinc status, healthy population

Mild-moderate (marginal) zinc deficiency is common in several developing countries, as the commonly staple foods have low zinc contents and are rich in phytates. We know that the high phytates content of cereal proteins decreases the availability of zinc, thus, the prevalence of marginal zinc deficiency is likely to be high in a population consuming large quantity of cereal proteins.[1,2] The Iraqi population, and the Arab world populations, are thought to be at high risk of marginal zinc deficiency, due to an increase preference to cereal proteins.[3] Several studies done in Baghdad have reported that the higher prevalence of marginal zinc deficiency among healthy population associated with a higher likelihood for the inadequate dietary zinc intake .Thus, we largely directed this work to determine the prevalence of zinc deficiency among a healthy population in our locality, Duhok population, in an attempt to identify population groups for whom zinc status and zinc supplementation may be a concern.

METHODS

This study was conducted at the Department of Clinical Biochemistry/

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School of Medicine/ Faculty of Medical Science-University of Duhok and Azadi General Teaching Hospital during the of period September 2009 to June 2010.A random sampling method was used to select a representative sample. A total of 332 subjects living in different areas of Duhok City were enrolled in the study. They were relatives of patients attending Azadi General Teaching Hospital, health workers and their relatives. A pre-tested questionnaire was designed to obtain information on gender, birth date, weight, height, residence, use of medications and minerals supplements, data on the number of household and number of rooms of the house. Crowding index, an indicator for socioeconomic status was calculated by dividing the number of household by the number of rooms of the house, presence of any coexisting morbidities were also recorded. The height and weight measurement for each subject was used to calculate the Body Mass Index (BMI) as a weight in kilograms (kg) divided by height in meter squared (m). Subjects with BMI less than 25 were considered normal, while those with BMI 25-29.9 were considered overweight and those BMI ≥ 30 were considered obese. [4] Inclusion criteria were apparently clinically healthy and absence of recent and/or chronic disease and not using mineral supplements. Marginal zinc deficiency and sub-optimal zinc status in the subjects studied were evaluated according to the cutoff values of serum zinc stated by others. [3,11] Values between 50-70 µg/dl were considered mild-moderate zinc deficiency. All subjects were asked to attend the Lab-Department of Clinical Biochemistry in Azadi General Teaching Hospital (Duhok) in the morning after overnight fasting for 12-14hours. Blood samples were collected between 9:00-11:30 a.m., and the serum was separated for zinc measurement. Zinc in serum was measured by direct enzymatic colorimetric method, using a commercial kit (Giesse Diagnostic, Italy);
All data were analyzed by using the Statistical Package for Social Science SPSS version 18.0; paired student t- test was used to assess differences in serum analyte among groups. Level of statistical significance (P value) was set at < 0.05.
Verbal consent was obtained from the study subjects or from their parents before enrollment in the study.

RESULTS

(Table 1) shows the mean values of serum Zinc concentration in different age groups (children, adolescents, adults and elderly people). Significantly lower mean values of serum Zinc concentrations were demonstrated in children and adolescents compared to other age groups (P<0.05 and p<0.01) respectively. (Table 2) shows the distribution of subjects in the studied groups according to serum Zinc levels. Of the three hundred thirty-two subjects, 10% (n=33) had mild-moderate zinc deficiency. Children and adolescents had a higher prevalence of zinc deficiency than adults.

Table1. Serum zinc concentration for studied subjects

	n	Age group	Serum zinc µg/dl Mean±SD	range
Children*	21	6-10 years	77.6±15.0	62.5-92.6
Adolescents**	26	11-19 years	68.3±15.2	53.1-83.5
Adults	256	20-65 years	82.1±15.5	66.5-97.6
Elderly people	29	>65 years	83.3±8.2	75.1-97.6
Total	332	6-80 years	81.1±14.8	53.1-97.6

* *Children Vs adults, p<0.05; **Adolescents Vs adults, p<0.01*

Table 2. Distribution of subjects in the studied groups based on serum zinc levels.

Subjects	Serum Zinc levels (ug/dl)	
	< 70 N(%)	>70 N(%)
Children	10(47.6)	11(52.4)
Adolescents	14(53.8)	12(46.2)
Adults	9(3.5)	247(96.5)
Elderly people	0(0)	29(100)
Total	33(10.0)	299(90)

Serum zinc <70ug/dl=mild-moderate zinc deficiency

(Table 3) shows the Mean+SD of serum zinc values by gender, residence, nutritional status and social status. Significantly lower mean serum zinc concentration values were observed in subjects with lower social status and in those living in rural areas. There was no significant gender difference in mean serum zinc concentration for all studied subjects. Subjects with normal weight had lower mean serum zinc concentration as compared to those with over weight or obese, but the difference was not significant (p=0.09).

Table 3. Mean±SD of serum zinc by gender, residency, nutritional status and social status.

Subjects	n	Serum Zinc (ug/dl) Mean±SD	p-value
Gender			
Males	**181**	**81.9±17.1**	**0.08**
Females	**151**	**79.6±13.5**	
Residency			
Urban	**201**	**83.7±15.4**	**<0.01**
Rural	**131**	**77.1±13.9**	
Nutritional status			
Normal Weight	**131**	**78.4±14.3**	**0.09**
Overweight	**138**	**81.1±15.9**	
Obese	**63**	**83.7±17.8**	
Social status*			
Low	**128**	**72.8±15.8**	**<0.01**
High	**204**	**81.4 ±20.9**	

* *Low social status (Crowding index 3.3 +1.9) Vs high social status (crowding index 2.1 +1.2).P<0.01*

DISCUSSION

Marginal zinc deficiency appears to be an important public health problem in many developing countries, including Iraq. [5] Previous data show that zinc deficiency is wide spread among children and elderly subjects. Our study revealed the prevalence of mild-moderate zinc deficiency was more among children and adolescents and the mean serum zinc concentration values lower than the values from adults and elderly people. This observation of high prevalence of low serum zinc concentrations in these children and adolescents does support the existence of marginal zinc deficiency in these populations. Indeed, pediatric populations and adolescents are known to be more sensitive to suboptimal zinc nutrients than adults perhaps because zinc is more important for normal growth and developments. [6] Al-Timimi et al also reported a high prevalence of mild-moderate zinc deficiency among children and adolescents in Baghdad population[3] It was reported that 89.9 percent of the

children had mild-moderate zinc deficiency and 54.7% of the population studied had such deficiency. The most striking finding of the present study was the appearance of lower incidence of mild-moderate zinc deficiency among the subjects studied compared with earlier data for children and adults in Baghdad City as well as other countries. [7,8,12] For example, in children less than ten years studied here, prevalence of mild-moderate zinc deficiency was lower (46.7%) as compared with those of the above mentioned study and the prevalence of mild-moderate zinc deficiency in all subjects studied was 10%. Such a difference is especially noteworthy because several factors are known to impact negatively on biochemical zinc status. Of these, dietary factors are with the most marked negative effect on serum zinc concentration. [10] Thus, the reduction in the prevalence of marginal zinc deficiency reported here appears to be associated, at least in part, with the general nutritional status of Duhok City population, which is highly affected by socioeconomic status. Although our observations show the low socioeconomic status affected serum zinc concentrations, but the majority of the subjects studied were with high socioeconomic status, good nutritional status and had higher serum zinc concentrations. The reason for this is probably related to high income associated with increases energy intake. However, the low serum zinc concentration in our rural subjects may be explained by higher consumption of rice and vegetables which may preclude adequate zinc absorption because of their high phytate and fiber content. [9]

It must be noted that the present study has limitations. Firstly, the study involved a small number of subjects with limited age and the results must be confirmed in a large sample. Secondly, dietray zinc intake was not used as variable in the present study. Even though the present study indicated that mild-moderate zinc deficiencies do exist among Duhok city population, especially in children and adolescents, and thus it necessitates the need for an effective public health intervention means to improve the zinc status of the population.

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پوختە

ئاستئ كەرستئ (زينك)ى لدەف خەلكئ ديار ساخلەم لباژيرئ دهوكئ, عيراق

باكگراوندا بابەتى: كيمبونا بەلافبونا كەرستئ (زينك)ى يا بەر بەلافە لهندەك دەولوتين باشڤەچوى, ژبەر كو ژيدەرئ وان يئ خوارنئ كيم (زينك) و گەلەك (فايتيت) يئ تيدا هەى, ويا زانيە كو (فايتيت) دابينكرنا (زينك)ى دناف لەشيدا كيم دكەت, ژبەر هندئ ئەف ڤەكولينە هاتە ئەنجامدان ژبو دەستنيشانكرنا ئاستئ (زينك)ى لدەف هندەك كەسين دەستنيشانكرى لباژيرئ دهوكئ, عيراق.

ريكين ڤەكولينئ: ئەف ڤەكولينە هاتە ئەنجامدان ل بەشئ كيميا ژيانى/فاكولتيا زانستين بژيشكى ل زانكويا دهوكئ لماوئ دناڤ بەرا ايلولا 2009 و خزيرانا 2010. ڤەكولينئ 332 كەسين ساخلەم بخوڤە گرتن (ژيئ وان دناڤبەرا 6 هەتا پتر ژ 65 سالا) كو دژين لهندەك جهين جودا جودا لباژيرئ دهوكئ. كاغەزەكا پسيارا هاتبو چيكرن بەر وەخت ژبو كومكرنا پيزانينا لسەر رەگەزى, ميژويا ژدايكبونئ, بلندى, كيشە, ئاكنجيبون, و ئاستئ جفاكى. بو هەر كەسەكى, ريژا (زينك)ى دناف خوينئ دا هاتە كيشان. ئەو كەسين زينك دناف خويناواندا كيمتر ژ (50 ug/dl) هاتنە هژمارتن كيم زينكى دناف خوينيدا ب رەنگەكئ ئاشكرا, وئەوين زينك (50-70 ug/dl) هاتنە هژمارتن كيم زينكى دناف خوينيدا ب رەنگەكئ كيم بو ناڤنجى.

ئەنجام: ريژا (زينك)ى دناف خوينئ كيمتربو ب رەنگەكئ ئاشكرا لدەف بجيكو سنيلەيا هەڤبەركرن دگەل گروپين ژيين دى ($P<0.05$ and $p<0.01$) لديف ئيك. ژ 332 كەسان,(n=33) 10% كيم زينكى دناف خوينيدا هەبوو ب رەنگەكئ كيم بو ناڤنجى. بچيك و سنيلا ريژەكا زيدەتر هەبوو يا كيم زينكيئ دناف خوينيدا هەڤبەنديكرن دگەل كەسين مەزن (47.6% and53.8% Vs 3.5%). كيم زينكيئ يا دژوار دناف خوينيدا نەهاتە ديتن لدەف چ كەسان. ب رەنگەكئ ئاشكرا ريژا زينكى دناف خوينيدا كيمتربوو لدەف ئەو كەسين ژئاستئ جڤاكى يئ كيم و ئەو كەسين دژين ل گوندا. جودايى نە ب رەنگەكئ ئاشكرابو ل تييكراييا ئاستيئ زينكى ل ناڤ خوينا لدەف هەروو رەگەزا. ئەو كەسين كيشا وان يا نورمال تێكراييا ئاستێ زينكى ل ناڤ خويناواندا كيمتربوو ژوان كەسين كيشا وان يا زيدە بەلئ جودايى نە ب رەنگەكئ ئاشكرابوو ($p=0.09$).

دەرئەنجام: كيمبونا ئاستئ زينكى دناڤ خوينيدا ب رەنگەكئ كيم بو ناڤنجى هاتە تيبينى كرن لدەڤ كەسين هاتينە فەكولين دگەل ريژەيەكا بەلاڤبونئ يا بلندتر لدەف زاروك و سنيلا, و ئەڤە پيدڤى ب ريكين ساخلەميا جڤاكى هەيە ژبو راستڤەكرنا ئاستئ زينكى لدەڤ خەلكى.

الخلاصة

حالة الخارصين الحالية لدى السكان الأصحاء في مدينة دهوك، العراق

الخلفية والأهداف: نقص انتشار الخارصين شائع في عدد من الدول النامية, لأن مصدر الغذاء لديهم يحتوي على كميه قليله من الخارصين وكميه عاليه من الفايتيت، والمعروف أن الاخيرة تقلل من توفر الخارصين, ولذلك هذه الدراسة أجريت لتحديد حالة الخارصين الحالية لدى مجموعه محدده من السكان الأصحاء في مدينة دهوك, العراق.

طرق البحث: تمت هذه الدراسة في قسم الكيمياء الحياتية/ سكول الطب/فكولتي العلوم الطبية في جامعه دهوك خلال الفترة ما بين أيلول 2009 و حزيران 2010. تضمن البحث 332 شخص (أعمارهم ما بين 6 الى أكثر من 65 سنه) يعيشون في مناطق مختلفة من مدينة دهوك. ورقة الاستبيان صممت من قبل لجمع المعلومات حول الجنس, تاريخ الميلاد, الارتفاع, الوزن, الاقامه, والحالة الاجتماعية, تم قياس مستوى الخارصين في الدم لكل شخص. اعتبر الشخص الذي لديه مستوى الخارصين اقل من (50 ug/dl) نقص الخارصين بشكل ملحوظ والذين لديهم (50-70 ug/dl) نقص الخارصين بشكل قليل إلى متوسط.

النتائج: مستوى الخارصين في الدم كان اقل بشكل ملحوظ في الأطفال والمراهقين مقارنة بالمجاميع العمرية الأخرى (P<0.05 and p<0.01) بالتسلسل. من الثلاثة مائة واثنان وثلاثون شخصا,(n=33) 10% كان لديهم نقص مستوى الخارصين في الدم بشكل قليل إلى متوسط. الأطفال والمراهقين كان لديهم نسبه أعلى من نقص انتشار الخارصين مقارنة بالبالغين (47.6% and53.8% Vs 3.5%). نقص انتشار الخارصين الشديد لم يتواجد في أي من الفئات العمرية. بشكل ملحوظ كان مستوى الخارصين في الدم قليلا في الأشخاص من ذوي الطبقة الاجتماعية الدونية وفي الأشخاص الذين يعيشون في المناطق الريفية. لم يكن الاختلاف بشكل ملحوظ في معدل القيمة لمستوى الخارصين في الدم بين الجنسين. الأشخاص من ذوي الوزن الطبيعي كان لديهم معدل القيمة لمستوى الخارصين في الدم اقل منه في الأشخاص ذات الوزن الزائد ولكن الاختلاف لم يكن بشكل ملحوظ (p=0.09).

الاستنتاج: نقص مستوى الخارصين في الدم بشكل قليل إلى متوسط لوحظ في الأشخاص الذين تمت دراستهم مع نسبة انتشار أعلى في الأطفال والمراهقين, وهذا يستلزم الحاجة لطرق تدخل صحة المجتمع لتحسين حالة الخارصين لدى السكان.

ISOLATION AND IDENTIFICATION OF *LEGIONELLA PNEUMOPHILA* FROM PATIENTS SPUTUM AND ANTIBACTERIAL ACTIVITY OF THE *GLYCYRRHIZA GLABRA* AND *MALVA NEGLECTA* ON IT

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ABSTRACT

Among 240 sputum samples , 20 isolates (8.3%) were identified as *Legionella pneumophila* in Hawler and Rizgary Teaching hospitals in Erbil city, by morphological, cultural, and biochemical characteristics. The pattern of resistance revealed that the highest resistance was for penicillin G, polymyxin B, vancomycin, while it was fully sensitive to azithromycin and clarithromycin, and it showed variable sensitivity to other antibiotics. The effect of the two medicinal plants (*Glycyrrhiza glabra* and *Malva neglecta*) on *L. pneumophila* growth was studied, the results showed that the activity of both plants by using MIC were 200, 300 µg/ml for aqueous extract and 250 , 400 µg/ml for alcoholic extracts respectively, while MIC was 300 µg/ml when extracts of both plants were used combined.

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Key words: *Legionella pneumophila*, Antibiotics, *Glycyrrhiza. glabra* and *Malva neglecta*

Legionella pneumophila was first recognized as a disease entity from a pneumonia outbreak in July 1976, the convention of the American legion in Philadelphia were struck down with a mysterious acute respiratory illness[1] . The primary isolation of *L. pneumophila* in laboratory is difficult, because it is fastidious and slow growing as well as it requires prolonged incubation period [2] .

During the last two decades, resistance to antimicrobial agents is a major global public health problem as well as the appearance of undesirable side effects of certain antibiotics and the emergence of previously uncommon infections led to search for new antibacterial compounds with improved activity to replace those that have become inactive is therefore necessary and traditional healers use many plants to treat diseases [3,4]. *Glycyrrhiza glabra* (Liouorice) which belong to family Leguminosae, is a tall, erect perennial herb with branched stalks with deep-rooted, long branch roots and stolons, brown on the external surface and yellow internally. It has a characteristic sweetish taste. Leaves are branches even pinnate. Flowers are hermaphrodite, bluish-purple. Fruits are Small smooth-skinned leguminous seedpods [(5)].The most important chemical constituents detected in liquorices roots were glycyrrhizin and flavonoid complexes [6]. The liquorice roots consist of carbohydrate substances, mineral salts, saponic substances and volatile oil. In addition, liquorice are known to enhance the elimination of mucus from the lung, therefore, it has been used to treat respiratory conditions such as bronchitis and it may also be used to soothe a sore throat[7,8]. *Malva neglecta* (Common mallow) belong to family Malvaceae which is annual short-lived perennial herb, stem branches sparingly to densely furnished, leaves orbicular-reniform, flowers white or lilac with pinkish veins, seeds brown [9]. The leaves of Common mallow contain gelatinous component, tannins, and vitamins A, B1, B2, and C; however, the flowers contain anthocyanin pigment [10,11]. The aim of the present study

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was isolation and identification of *L. pneumophila* from sputum and studying the susceptibility and resistance of the isolates to a number of antibiotics. Detection of the inhibitory effect of some medicinal plants on the growth of the isolates.

METHODS

Collection of samples

A total of two handred forty sputum specimens were collected within four months (November 2005 to March 2006) from the patients in Hawler and Rizgary Teaching hospitals in Erbil , specimens collected at first morning in a sterile cup, then immediately transferred to laboratory and subjected directly to microbiological examinations.

Isolation, characterization and identification of *L. pneumophila*

Different culture media were used to isolate this pathogen and all of these media were supplemented with iron salts and L - cysteine HCl which are growth factors for cultivation of bacteria, also all culture media were modified by using ferric nitrate in place of ferric pyrophosphate as a source of iron in concentration 0.25g/L [11].

- Charcoal Yeast Extract Hemoglobin Agar Medium :- prepared as previously described by[13] .
- Modified Wadowsky Yee Medium (MWY):- prepared as described by [14].
- Charcoal Yeast Extract with Dyes Medium: - prepared as previously described by [15].
- Buffered Charcoal Yeast Extract (BCYE) Selective Agar/ Glycin, Polymxin B, Vancomycin, Nystatin (GVPN) Medium:- Selective medium consist of Buffered Charcoal Yeast Extract Agar Medium (BCYE) which prepared as previously described by [12] supplemented with:-

Glycine	30.0g
Polymyxin B	80.0U
Vancomycin	1.0µg
Nystatin	100 µg

- Buffered Charcoal Trypton Agar Medium: - This medium was prepared such as BCYE agar but here trypton was used in place of yeast extract.

Each isolate was tested by gram staining with elongation period of safranin from 30 seconds to 10-15 minutes because *L. pneumophila* is weakly stained [15].All biochemical tests (Table 1) were carried out on suspected colonies according to[17-19].

Detection of Extended Spectrum β-Lactamase (ESBLs) of L. pneumophila

To investigate (ESBLs) enzyme, the method of disc approximation was used as mentioned by [20].

Antibiotic Sensitivity

The modified Kirby – Bauer method of[21] was carried out to determine the susceptibility of *L. pneumophila* to 21 antibiotics .

Preparation of plant extracts

Roots of Gl. glabra was obtained from AL –Hadder pharmacy, Baghdad (voucher number 5/305 in 1991).While, leaves, flowers, and seeds of the *M. neglecta* were collected from different places of Erbil city, then classified in the Education Salahaddin University Herbarium (ESUH).

To prepared aqueous extraction of both plants by using the method [22] .Alcoholic extractions of the *Gl. glabra L. and M. neglecta* were done by the modified method of [23].

Determination of antimicrobial activity by using Minimum Inhibitory Concentration (MIC)

Different concentration were prepared (10, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800)µg/ml separately and combination for both aqueous and alcoholic extracts, 10ml of sterile Yeast extract broth were added for each concentration and used as control samples, the bacterial growth was evaluated on the basis growth curve and turbidity of the suspension which all tubes

were read by Spectrophotometer at 600nm[24].

RESULTS AND DISCUSSION

Among 240 sputum samples; twenty (8.33%) isolates were identified as *L. pneumophila*, this result was in agreement with that previously reported by[25] who demonstrated that 74 sputum samples 7(9.5%) specimens yielded *L. pneumophila*. This percentage is relatively low and this might due to that the isolation of Legionella from sputum is more difficult because of the predominance normal bacterial flora [26]. Numerous studies have shown that the incidence of nosocomial pneumonia by *L. pneumophila* is dependent on many factors including cigarette smoking, heavy alcohol drinking, and advanced age included age, male gender and immuno-compromised patients [27]. Other studies have linked nosocomial legionellosis to potable water and air conditioning system and cooling towers [28]. Other researchers revealed that the patients receiving respiratory therapy with potentially contaminated medical equipments or whose care includes the use of aerosol generators such as humidifiers are clearly at increased risk for contracting nosocomial legionnaires disease [27,29]. A number of laboratory preparing media were used in addition to modification of these media by addition of ferric nitrate instead of ferric pyrophosphate and all the isolates incubated at 37ºC and 2.5-5% CO2 for 7 days :-

Charcoal Yeast Extract Hemoglobin Agar Medium was similar to BCYE agar except the hemoglobin (1%) was used as source of iron instead of ferric nitrate, iridescence grayish-white colonies with cut-glass appeared and these results were in accordance with those previously reported by [30] (Figure 1).

On Modified Wadowsky Yee Medium (MWY) Colonies appeared as green iridescence with cut-glass shape as it was show in (Figure 2) and these results were in accordance with that previously reported by [31] , they mentioned that this medium was BCYE agar with antimicrobial agents such as Polymyxin B (that inhibits the growth of gram negative bacteria), Vancomycine (inhibits the growth of gram positive bacteria) and Anisomycin (which inhibits the growth of yeast and fungi), and differential dyes such as bromothymol blue and bromocresol purple. In addition to amino acid glycine which sufficiently inhibits the non-Legionellaceae bacteria and as a result greatly facilitated the isolation of *L. pneumophila* from specimens [14].

On Charcoal Yeast Extract with Dyes Medium produced green iridescence colonies of *L. pneumophila* (Figure 3), and this confirmed by the results reported by [15] , they illustrated that the addition of bromothymol blue and bromocresol purple were proven as an aid for the identification and classification of *L. pneumophila*.

On BCYE Selective Agar/ GVPN Medium *L. pneumophila* appeared as iridescence grayish-white colonies (Figure 4). Nystatin was an antifungal agent and an inexpensive alternative to anisomycin in the formulation of selective BCYE agar media.

On Buffered Charcoal Trypton Agar Medium Yeast extract was replaced by trypton, and it was found that the growth of *L. pneumophila* on this medium was identical to that obtained on the BCYE agar medium (Figure 5) and these results are supported by[30].

Each isolate was non-spore forming, non capsulated and poorly-stained gram negative rods (Figure 6), these results were agreed with that published by (26). (Table 1) showed the biochemical characteristics of *L. pneumophila* and these results were agreed with that reported by other studies [26,32].

During this study it has been found that [15] 75% of the isolates were resistant to Augementen disc (amoxicillin-clavulinic acid), cefotaxim, piperacillin, and ceftazidime antibiotics, the combination of

antibiotics leads to an increase in the activity of antibiotics, and the mixing of amoxicillin and clavulanic acid called augmenten caused decrease of MIC for these antibiotics and become more active against the tested bacteria (Roy et al., 1989). Furthermore, a wide using of antibiotics by the patients may facilitate the spread of bacteria which produced ESBLs enzymes [34] .

(Figure 7) and (Table 2) revealed that the azithromycin and clarithromycin had highest effective against L. pneumophila isolates with a percentage (100%) for both antibiotics, in the second grade doxycyclin hydrochlorid, ciprofloxacin, neomycin, norfloxacin, erythromycin, tetracycline, trimethoprim, chloramphenicol and kanamycin were (95%, 90%, 70%, 85%, 70%, 70%, 70%, 60%, and 60%) respectively, which showed good effectiveness against tested isolates. Furthermore, [35] studied the activity of antibiotics against 82 isolates of *L. pneumophila* and they found that all isolates were susceptible to clarithromycin, erythromycin and ciprofloxacin. Newer macrolides such as azithromycin and clarithromycin in addition to fluoroquinolones such as ciproflaxacin and norfloxacin have become an important alternative to macrolides antibiotics in the treatment of Legionnaires disease because if compared to erythromycin, they have a broad antibacterial spectrum [36]. On the other hand, the results showed that the percentage of resistance to vancomycin, polymyxin B, and penicillin was (100%), while it was (95%, 85%,80%, 75%, 75%,70%, and 60%) to ampicillin, cephalothin, carbencillin, clindamycin, streptomycin, lincomycin, and ceftazidine respectively, and these results were in accordance with those reported by [37]. vancomycin is a glycopeptide that has no usefulness against gram negative bacteria, while polymyxin B is a bactericidal antibiotic that is effective against gram negative bacteria which causes injury to the plasma membrane, but *L. pneumophila* was resistant to these antibiotics , might be due to that *L. pneumophila* posses a special type of lipopolysuccharid which prevents the permeability of the antibiotics [38] . In fact, high resistance to pencillin G and ampicillin due to the ability of of *L. pneumophila* to produce β-Lactimase enzyme [39] .

The present study showed that the MIC for aqueous extract of *Gl. glabra* and *M. neglecta* were 200, 300 µg/ml respectively and 250,400 µg/ml for alcoholic extract respectively. On the other hand, the MIC of aqueous and alcoholic extracts of both plants was 300 µg/ml this results were similar to those obtained by [3]. The activity of liquorice is attributed to the presence of glycyrrhizin which has been shown to stop the growth of many bacteria and it was believed to be responsible for the plant action in the respiratory system, acting as an expectorant and helped to prevent and ease coughing; this ingredient and its derivatives work as anti inflammatory and allergenic effects, especially when treating asthma. In human body the glycyrrhizin is hydrolysed to glycyrrhetinic acid, which has triterpenoid structure [40] . In addition, many studies reported previously that some antimicrobial flavonoid and several bioactive phenolic compounds were formed in liquorice roots [41].Common mallow plants were widely used by local people as food and to treat different disease such as bronchitis and to prevent throat infections [42]. Furthermore, [43] indicated that common mallow leaves and roots were used as antibacterial agents which inhibit the growth of many gram negative bacteria. Indeed, all parts of the plant induce removal (coughing up) of mucous secretion from the lungs or taken internally in the treatment of respiratory system disease or inflammation of the digestive or urinary systems [44,45]. However, [8] found that the activity of Malva sp. may be due to tannins, vitamins, salicylic and phenol compounds which were used as antibacterial agents. Moreover, [46] reported that the medicinal

plants had inhibitory effect on the growth of *L. pneumophila* .

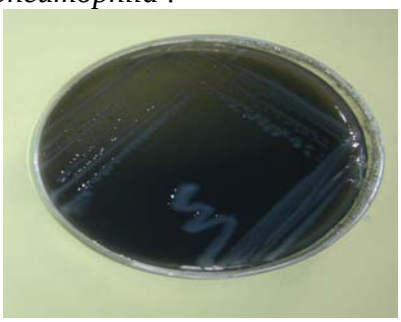

Figure 1. Growth of *L. pneumophila* on Charcoal Yeast Extract Hemoglobin Agar Medium .

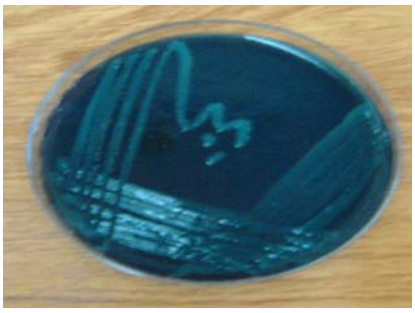

Figure 2. Growth of *L. pneumophila* on MWY Agar Medium.

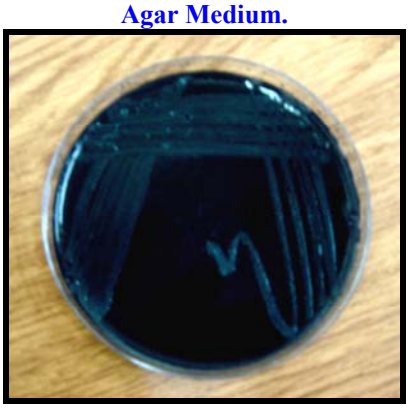

Figure 3. Growth of *L. pneumophila* on Charcoal Yeast Extract Agar with Dyes.

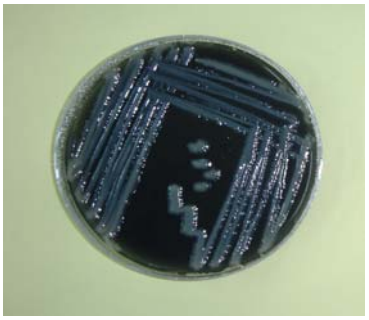

Figure 4. Growth of *L. pneumophila* on BCYE Selective Agar / GVPN Medium.

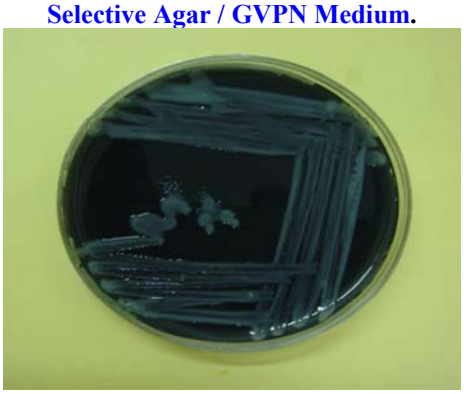

Figure 5. Growth of *L. pneumophila* on Buffered Charcoal Trypton Agar Medium .

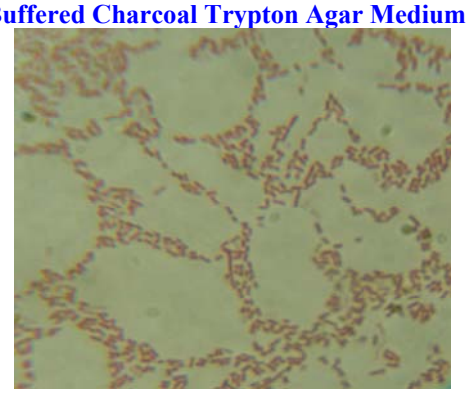

Figure 6. *L. pneumophila* cells stained with Gram stain (1000X).

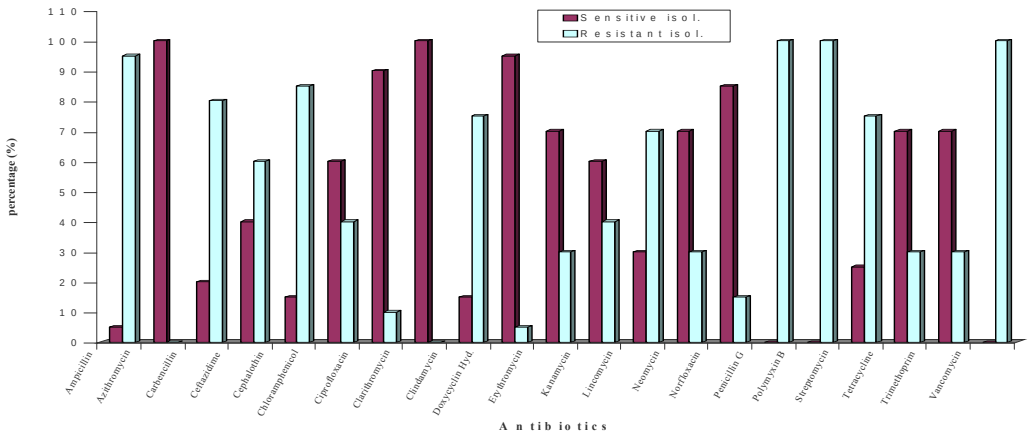

Figure 7. Antibiotic sensitivity of *L. pneumophila* isolates.

Table .1 Results of biochemical tests for *L. pneumophila* bacteria under the study.

Biochemical tests	Results of clinical isolates
Oxidase test	+ (100%)
Catalase test	+ (100%)
Gelatinase test	+ (100%)
Hippurate hydrolysis test	+ (100%)
Starch hydrolysis	+ (85%)
DNase test	+ (95%)
Lipase and Lecithinase test	+ (100%)
Protease test	+ (95%)
Urease test	–
Nitrate reductase	–
Motility test	+ (100%)
IMViC test	–
B-Lactamase	+ (100%)

(+): Positive (-): Negative

Table 2. Antibiotic sensitivity of clinical and environmental isolates of *L. pneumophila*.

Antibacterial antibiotics	Disc code	Concentration .(µg/ disc)	Sensitive isolates		Resistant isolates	
			NO.	%	NO.	%
Ampicillin	AM	10	1	5	19	95
Azithromycin	AZM	15	20	100	-	-
Carbencillin	PY	100	4	20	16	80
Ceftazidime	CAZ	30	8	40	12	60
Cephalothin	KF	30	3	15	17	85
Chloramphenicol	C	30	12	60	8	40
Ciprofloxacin	CIP	50	18	90	2	10
Clarithromycin	CLR	15	20	100	-	-
Clindamycin	DA	2	5	15	15	75
Doxycyclin Hydrochloride	DO	30	19	95	1	5
Erythromycin	E	15	14	70	6	30
Kanamycin	K	30	12	60	8	40
Lincomycin	L	2	6	30	14	70
Neomycin	N	30	14	70	6	30
Norfloxacin	NOR	10	17	85	3	15
Penicillin G	P	10IU	-	-	20	100
Polymyxin B	PB	300IU	-	-	20	100
Streptomycin	S	10	5	25	15	75
Tetracycline	TE	30	14	70	6	30
Trimethoprim	TMP	5	14	70	6	30
Vancomycin	VA	30	-	-	20	100

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پوختە

جیاکردنەوە و دەست نیشان کردنی بەکتریای *Legionella pneumophila* ل بەغلەمی نەخوشان و دژە زیندەکی.کاری گەری هەندێ رووەکی پزیشکی وەکو پەگی مەکووك *Glycyrrhiza glabra* و تۆلکە *Malva neglecta*لە سەر بەکتریای *L.pneumophila*.

ئەم لێکۆلینەوەیە بریتی یە لە جیاکردنەوە و دەست نیشان کردنی (20)بەکتریای Le*gionella pneumophila* بەرێژەی (8.3%)لە (240)نموونەی کۆکراوە لە بە لغەمی نەخوشەکانی نەخۆشخانەی هەولێر و رزگاری فێرکردن لەهەولێر .هەموو جیاکراوەکان دەست نیشان کران بەپێ تاقیکردنەوەی سیفاتی مۆرفۆلۆجی و کێلگەی وروالەتی کیمیاوی زیندەوەری .هەستیاری بەکتریا جیاکراوەکاوەن بەدژە زیندەکی بە بەکارهێنانی (21)دژەزیندەکی پشکێنران وئەنجامەکان دەریان خست کە هەموو جیاکراوەکان بەرگریەکی تەواویان نیشان دا بۆ هەندێ دژە زیندەکی وەکو Vancomycin, polymyxin B, Penicillin G, کە چی بە تەواوی هەستیار بوون بۆ Azithromycinو Clarithromycin، وە هەروەها گۆراوە هەستیاریان نیشان دا بۆ هەندێ دژە زیندەکی.کاری گەری هەندێ رووەکی پزیشکی وەکو پەگی مەکووك *Glycyrrhiza glabra* تۆلکە *Malva neglecta*لە سەر بەکتریای *L.pneumophila*لێکۆڵرایەوە،ئەنجامەکان دەریان خست کە چالاکی هەردوو رووەکەکە بە MICکە بریتی بوون لە (200 ، 300) مایکرۆگرام/مل یەك لە دوا یەك بۆ پالاوتە ئاوییەکان وە (250 ، 400) مایکرۆگرام/مل بۆ پالاوتە ئەلکهولیەکان، بەلام MIC بریتی بوو لە (300) مایکرۆگرام/مل کە هەردوو رووەکەکان بە یەکەوە بەرکاهێنران.

الخلاصة

عزل وتعريف البكتيريا *Legionella pneumophila* من بلغم المرضى والنشاط المضاد للبكتيريا لنباتات عرق السوس *Glycyrrhiza glabra*و الخباز *Malva neglecta* عليه

تضمنت الدراسة عزل وتشخيص (20)عزلة وبنسبة (8.33%)جرثومة *Legionella pneumophila*من (240)عينة من قشع المرضى في مستشفى هولير ورزكاري في مدينة اربيل .تم تشخيص جميع العزلات اعتمادا على صفاتها الشكلية والمزرعية والصفات الكيمياء الحيوية.كما اختبرت حساسية الجرثومة للمضادات الحيوية وباستخدام (21)مضادا حيويا، وقد اظهرت النتائج امتلاك جميع العزلات مقاومة مطلقة لبعض من المضادات مثل Penicillin G, Polymyxin B, Vancomycinوحساسية مطلقة لمضادات Azithromycimو Clarithromycin، وقد اختلفت في مقاومتها وحساسيتها للمضادات الاخرى .درس ايضا تاثير بعض النباتات الطبية عرق السوس *Glycyrrhiza glabra*و الخباز *Malva neglecta*على جرثومة *L. pneumophila*، وقد اظهرت النتائج فعالية كلا النباتين باستخدام MIC والتي بلغت (200، 300) مايكروغرام /مل على التوالي لكلا النباتين كل على حدة للمستخلص المائي (250 ، 400)مايكروغرام /مل على التوالي للمستخلص الكحولي في حين بلغت (300) MICمايكروغرام /مل عند استخدام كلا النباتين مع بعضها.

NATURAL STAINS AS A SUBSTITUTE OF SYNTHETIC STAINS

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ABSTRACT

Background and Objectives: Polymethyl methacrylate (PMMA) is currently the material of choice for denture base fabrication, that it has the advantages of good pigment wettability, high gloss and so on.
Uses of natural stains (amaranth and vanilla) cheap and available in our country instead of the synthetic stains with heat cure acrylic resin denture base material for matching with natural human gingiva. Materials and **Methods:** Ninety samples were prepared of pink heat cure acrylic resin Vertex^{TM} material, fifteen samples with natural additives and seventy five samples with synthetic Vertex acrylic stain^{TM}.. The color of samples and gingival color of 24 healthy young participate were measured by Vita Easyshade device then compared between them. Statistical analysis was done by special designed program prepared for this study in Mat lab program 2010 that calculate the color difference between all the measurements that appeared only the (ΔE) that ≤ 6.8. **Results:** Results showed that (ΔE) ≤ 6.8 between some of natural and synthetic stains matched in relation to color of patients' gingiva, and some natural stains matched the color of the patients' gingiva. Conclusions: The results approved can be using the natural stains vanilla and amaranth instead of the synthetic Vertex acrylic stains that is clinically acceptable compared in relation to patients.

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Key words: Natural stains, Vanilla, Amaranth, PMMA.

Polymethyl methacrylate (PMMA) is currently the material of choice for denture base fabrication [1], that it has the advantages of good pigment wettability, high gloss and so on [2].

In order to obtain a natural looking restoration, there are two crucial steps in everyday dental practice; the selection of color through shade guide which will harmoniously integrate itself with surrounding biological tissue and consequently the correct reproduction of the color in the prosthesis [3]. Healthy gingival color ranges from pale pink and pink to dark red or purple [4].

Esthetic effects are sometimes produced in a restorative material by the incorporation of colored pigments; is pigments in non metallic materials such as resin, composites, dentures acrylics, silicone maxillofacial materials and dental ceramics[5].

Synthetic stains are mainly chemical products, which have some toxicity[6]. The obtainment of coloring matter based on natural products is of considerable importance since the United States have banned the use of synthetic coloring in foods [7].

Grain Amaranth (Amaranthus paniculatus) [8] it is a natural stain that the anthocyannin is the reddish pigments in amaranth flour and vegetation appear to have great potential as a source of natural, non–toxic red dyes, this pigment is used in food industry [9].

Natural vanillin (4-hydroxy-3-methoxybenzaldehyde) is one of the most common flavor chemicals and is used in a broad range of flavors [10,11]it is a tropical orchid belonging to the family Orchidaceae [11].

Color difference (ΔE) as value for color differences became a critical in color

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science as well as in industries ranging from textiles to dentistry that calculated as following (12):

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$
$$\Delta E = [(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2]^{1/2}$$

Vita EasyShade device is one that used For spectrophotometer measurement, this instrument provide data obtained over the range of visible wavelengths about 400 to 700 nm, captures the tristimuli HCL and subsequently calculates the values of L*, a* & b*[13].

METHODS

One type of pink heat-cured acrylic resin denture base material Vertex ™ type was used with natural (amaranth and vanilla), and synthetic stain ™ additives. The samples were prepared follow the recommended manufacturers' instruction powder/ liquid ratio. Curing was carried out by using conventional water bath methods in which placing flask in a thermostatically controlled water bath for curing cycle 1.5 hours at 74oC followed by 30 minutes at 100oC [14]. All prepared samples were stored in distilled water at 37oC for 7 days for conditioning before testing. Ten samples were prepared to the uniform size in dimension (30x20x1.5) ±0.03 mm (length, width and thickness respectively) [15], samples were divided into two groups:

First Group: Fifteen samples of natural stain additives type as follows:

Five samples with amaranth stain 0.1% wt/wt.

Five samples with vanilla additives 10% wt/wt.

Five samples with mixture of amaranth 0.02% and vanilla 0.08% respectively wt/wt.

Second Group: Seventy five samples prepared with synthetic stain™ additive types:

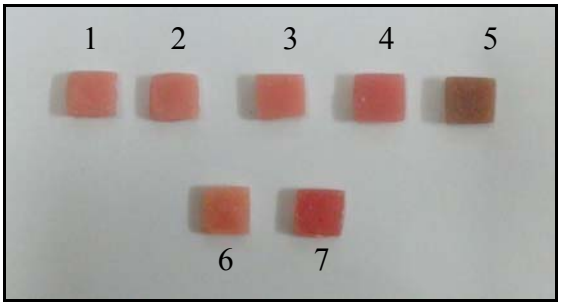
Fifteen samples of vertex acrylic stain™no.210 (1%, 5%, and 10% wt/wt).

Fifteen samples of vertex acrylic stain ™ no.220 (1%, 5%, and 10% wt/wt).

Fifteen samples of vertex acrylic stain ™ no.230 (1%, 5%, and 10% wt/wt).

Fifteen samples of vertex acrylic stain™ no.240 (1%, 5%, and 10% wt/wt).

Fifteen samples of vertex acrylic stain™ no.250 (1%, 5%, and 10% wt/wt) as in (Figure 1).



(1): Samples with synthetic stain No.210 additives, (2): Samples with synthetic stain No.220 additives, (3): Samples with synthetic stain No.230 additives (4): Samples with synthetic stain No.240 additives (5): Samples with synthetic stain No.250 additives, (6): Samples with vanilla additives and (7): Samples with amaranth additives.

Figure 1. Samples of synthetic and natural stain additives.

Color measurements were repeated five times for each sample and the mean of L*,a* and b* were calculated. Twenty four male (12) and female (12) dental students with age range 24±1 year. Mean of three measurements of gingival color was evaluated in the anterior region (in midpoint between free gingiva and deepest point of sulcus in central and lateral incisor regions) as in (Figure 2).

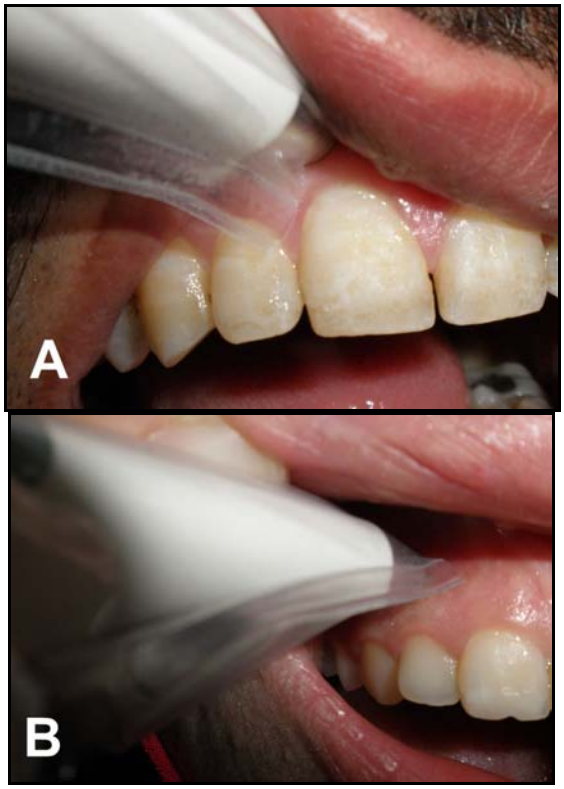


Figure 2. Measuring gingival color at (A); Central incisor region, (B); Lateral incisor region.

Figure 3. Easyshade device.

Measurement color matching: (CIE L*a*b*) color difference metrics were used for the performance analysis. Measurements were done by Vita Easyshade device that showed in Figure (3) to obtain the baseline L*, a*, b* values. The information from Dr.- lng. Wolfgang Rauh , a director Business Unit – Dental Devices in VITA company "can be use Easyshade to compare different samples of resins just be comparing the reported values but that the values will not correspond to values provided by other devices and similar to resins it be possible to use it to compare different gingiva but the values will not be absolute values")(16). The total color change (ΔE) between values was calculated for each pairs evaluated using the formula:

$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$

$\Delta E = [(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2]^{1/2}$

In principle, when $\Delta E \leq 3.7$(17) the difference in color between the paired values matched are acceptable in vitro and when $\Delta E \leq 6.8$(17) the difference in color between the paired values matched are acceptable in vivo, so the two samples are nearly have same color in vivo, when color difference be detected is > 6.8 that is not acceptable and the paired samples are not matched in vivo.

Statistical analysis carried out using a special program designed in Matlab 2010 program for this study to calculate (ΔE) between every value with all others and output appears only the paired that have $(\Delta E) \leq 6.8$.

RESULTS

The three values (L*a*b*) of the color measuring of all the prepared samples are listed in (Table 1). The value of the color measurement of gingival part of the oral cavity of patients are listed in (Table 2). (Table 3) listed the color differences between the color of the prepared samples and the color measurement of gingival part of the oral cavity of patients.

DISCUSSIONS

Results from the (Tables 1 and 2) show that (ΔE) of the patient versus prepared samples with natural stain vanilla additives in 10% is acceptable and the (ΔE) of the patient versus samples with synthetic stain no. 240 in 10% was also acceptable so the natural stain vanilla could be used instead of the synthetic stain no.240.

The color of the patients gingiva was matched the color of the (prepared samples with combined both amaranth 0.01% and vanilla 0.09%) and (prepared samples with amaranth stain 0.1% additives stain) as listed in (Table 3), so it could be applied in clinical uses.

Synthetic stain^{TM} no.250 in 1% with synthetic stain^{TM} no.250 in 5% and synthetic stain^{TM} no.230 in 5% with synthetic stain^{TM} no.230 in 10% matched he same patient gingival color so increase the stain percentage from 1% to 5% and from 5% to 10% not causing a significant color changes that should increase the percentages of the stain to cause additional color and this is a waste of the material and consequence higher the cost.

Table 1. L, a and b, Mean of Three Values of the Prepared Samples with Natural and Synthetic Stain Additives.

Prepared samples	L	a	b
P V (10 %)	53.44	27.28	12.04
P A (0.1 %)	41.6	35.58	15.3
P 9.9+ A 0.01 + v0.09 (1%)	42.16	38.26	17.7
P st1 (1 %)	51.78	29.92	11.92
P st2 (1 %)	52.08	29.86	11.28
P st3 (1 %)	51.9	30.22	11.4
P st4 (1 %)	54	30.26	13.12
P st5 (1 %)	49.12	27.32	10.84
P st1 (5 %)	54.1	28.26	11.06
P st2 (5 %)	57.16	28.02	12.06
P st3 (5 %)	53.9	30.92	12.72
P st4 (5 %)	53.84	33.04	13.9
P st5 (5 %)	43.78	24.14	11.74
P St1 (10 %)	55.2	25.7	10.06
P St2 (10 %)	57.16	26.86	11
P St3 (10 %)	55.12	30.42	14.04
P St4 (10 %)	49.94	31.68	12.92
P St5 (10 %)	44.02	18.78	12.84

L=lightness, a=redness and greenness, b=yellowness and blueness, P=pink acrylic powder, v=vanilla, A=amaranth, St1- 5= synthetic stain no.210–250.

Amaranth has been used for millennia to color everything to the rouge painted cheeks of South African women as it is red dyes[18], it used in dental field as additive to the dental wax[19]. Vanilla uses are mainly focused on uses in food industries, also it one commonly used remedy that has widely been reported to soothe toothaches when pain killers[20], and incorporated in the mouth rinse[21], but it's uses in dental field are not widely. Amaranth and vanilla stain are cheap and available in my country. This is a recent study of these natural stains (amaranth and vanilla) added to heat cure acrylic resin denture base as coloring agents and there is no similar previous study.

CONCLUSIONS

The results appeared that it can be using the natural stain (vanilla 10% wt/wt and amaranth 0.1% wt/wt) instead of the synthetic Vertex^{TM} acrylic stain compared in relation to patients that the color difference were evaluated with(CIE L*a*b*) system. The results appeared that ΔE between tested values is $\leq$ 6.8 that is matched clinically.

Table 2. The Mean Values of Color Measuring of the Patient's Gingiva.

patients	Gender	Area	L	a	b
1	f	Region a	58.2	19.5	25.6
1	f	Region b	53.7	28.0	27.0
2	f	Region a	50.8	27.2	21.6
2	f	Region b	59.7	27.6	26.9
3	f	Region a	38.9	26.7	25.3
3	f	Region b	44.2	25.1	29.6
4	f	Region a	57.5	16.5	25.6
4	f	Region b	67.0	10.6	28.3
5	f	Region a	64.7	10.2	25.9
5	f	Region b	45.4	29.1	19.3
6	f	Region a	76.2	3.0	25.8
6	f	Region b	50.4	26.7	15.2
7	f	Region a	52.9	19.0	23.8
7	f	Region b	48.9	23.2	13.6
8	f	Region a	69.5	7.2	50.1
8	f	Region b	5.8	19.8	28.4
9	f	Region a	50.8	17.1	25.3
9	f	Region b	51.7	17.6	36.3
10	f	Region a	52.9	16.3	21.8
10	f	Region b	55.0	19.5	31.9
11	f	Region a	47.4	25.2	21.7
11	f	Region b	55.6	16.2	38.5
12	f	Region a	53.5	24.1	28.0
12	f	Region b	71.0	12.9	31.6
13	m	Region a	42.6	36.0	20.2
13	m	Region b	57.2	14.8	27.2
14	m	Region a	59.3	16.7	23.5
14	m	Region b	53.7	25.8	24.8
15	m	Region a	40.2	33.7	17.8
15	m	Region b	54.4	22.7	24.7
16	m	Region a	61.7	16.0	28.7
16	m	Region b	46.2	31.6	21.3
17	m	Region a	56.9	17.1	28.4
17	m	Region b	63.4	15.5	29.6
18	m	Region a	66.0	12.1	31.2
18	m	Region b	54.7	22.4	24.2
19	m	Region a	60.4	15.2	35.5
19	m	Region b	55.1	15.2	35.5
20	m	Region a	58.6	16.3	25.9
20	m	Region b	52.4	26.5	22.4
21	m	Region a	54.8	19.1	28.8
21	m	Region b	57.2	15.2	27.2
22	m	Region a	74.0	6.6	34.9
22	m	Region b	60.5	16.6	28.2
23	m	Region a	53.8	22.4	30.4
23	m	Region b	57.2	17.3	36.8
24	m	Region a	52.3	20.7	25.5
24	m	Region b	57.3	18.2	29.6

L=degree of lightness, a=degree of redness and greenness, b=degree of yellowness and blueness, m=male, f=female, region a= central incisor region, region b = lateral incisor region.

Table 3. (ΔE) of the Samples that is ≤ 6.8.

Compared values	ΔE
13ma v P 9.9 + A 0.01 + v0.09 (1 %)	3.398706
15ma v P A (0.1 %)	3.42701
6fb v P V (10 %)	4.423076
6fb v P st5 (1 %)	4.586109
6fb v P st1 (1 %)	4.799083
7fb v P st5 (1 %)	4.96391
6fb v P St4 (10 %)	5.496399
7fb v P st5 (5 %)	5.527893
6fb v P st3 (5 %)	6.017375
6fb v P st3 (10 %)	6.120654

L=lightness, a=redness and greenness, b=yellowness and blueness. P=pink acrylic powder, v=vanilla, A=amaranth, St 1- 5 = synthetic stain no.210-250, 1–15=patients.

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پوختە

رەنگ کەرێن سروشتی وەك پێگهورەك بو رەنگ کەرێن دەستکرد

پێشەکی و ئارمانج: کەرستێ پولی مەتیل مێتاکریلێت PMMA نوکە دهێتە بکارئینان وەك کەرستێ هەلبژارتی بو بجهکرنا ددانێن دەستکرد کو گەلەك ساخلەتێن باش هەنە. بکارئینانا رەنگەرێن سروشتی (ئەمارانت و ڤانێللا) کو د ئەرزانن و دمشەنە ل وەلاتێ مە لجهێ رەنگکەرێن دەستکرد بو گونجاندن دگەل پدیا مروڤی یا سروشتی ئارمانجا ڤێ ڤەکولینێ بوو.

رێکێن ڤەکولینێ: 90 نمونە هاتنە حازرکرن ژ کەرستێ ئەکریلیك، و 15 ژوان کەرستێن سروشتی لسەر هاتنە زێدەکرن و 75 بێن **دەستکرد.** رەنگێ نمونا دگەل رەنگێ پدیا 24 گەنجێن ساخلەم کو بەشداربووین هاتە هەلسەنگاندن برێکا ئامیرێن تایبەت. پروگرامەکێ تایبەت یێ شروگەکرنا پێزانینا هاتە حازرکرن بو ڤێ ڤەکولینێ ژبو دیارکرنا جیاوازیێن رەنگی دناڤبەرا هەمی نمونان دا.

ئەنجام: ڤەکولینێ دیارکر کو جیاوازی هەبوو دناڤەرا رەنگکەرێن سروشتی و رەنگکەرێن دەستکرد لدەمێ گونجاندنێ دگەل رەنگێ پدیا بەشداربوویا، هەروەسا هندەك رەنگکەرێن سروشتی دگونجای بوون دگەل رەنگێ پدیێ.

دەرئەنجام: ژئەنجامان دوپاتبوو کو دشیاندایە رەنگکەرێن سروشتی (ڤانێللا و ئەمارانت) بهێنە بکارئینان لجهێ رەنگکەرێن دەستکرد (ڤێرتێکس ئئەکریلیك) کو جهێ رازیبوونا نەخوشان ژی یە.

الخلاصة

الملونات الطبيعية بدلا عن الملونات الاصطناعية

خلفية البحث والهدف: يعتبر الراتنج الاكريلي المادة الرئيسية في صناعة قاعدة طقم الأسنان, الذي يمتلك صفة اللمعان وقابلية التلوين و تهدف الدراسة الحالية إلى استخدام الملونات الطبيعية (الفانيلا والأمرانث) بدلا عن استخدام الملونات الاصطناعية مع ماده راتنج اكريل طقم الأسنان حراري التصلب والتي تقلل التكلفة والتأثيرات السمية للملونات الاصطناعية.

المواد وطرق البحث: تم تحضير تسعون عينه من راتنج اكريل الحراري, منها خمسه عشر عينه مضافا لها الملونات الطبيعية وخمسه وسبعون عينه مضافا لها الملونات الاصطناعية و بتراكيز مختلفة. ولتقيم صفه اللون تم استخدام جهاز (Easyshade) لقياس لون الجزء الأمامي لأربعة وعشرون مريضا ثم مقارنتها مع لون جميع العينات المحضرة. التحليل الإحصائي تم بتصميم برنامج خاص لهذه الدراسة باستخدام برنامج Matlab 2010 والذي يحسب فرق اللون بين كل زوج من العينات المحضرة ويظهر المتطابقة منها فقط والتي قيمتها تساوي أو اقل من 6,8.

النتائج: أظهرت النتائج بأنه اللون الناتج عن أضافه الملونات الطبيعية طابق لون اللثة لبعض المرضى. الاستنتاجات: بالإمكان استخدام الملونات الطبيعية(الفانيلا والأمرانث) بدلا عن الملونات الاصطناعية وذلك لمطابقة اللون مع لون لثة المرضى وهذا يقلل من التكلفة والتأثيرات السمية الناتجة عن استخدام الملونات الاصطناعية.

SUB ESCHAR INFILTERATION OF EPINIPHRINE IN EARLY EXCISION AND SKIN GRAFTING TO DECREASE BLOOD LOSS IN BURN SURGERY

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ABSTRACT

Background and objectives One of the problems in primary tangential excision of bum wounds is bleeding. To reduce bleeding, epinephrine solution used is composed of 1000 ml normal saline, with an added I ml 1/1000 aqueous epinephrine hydrochloride (1 mg epinephrine hydrochloride/1000 ml solution , 1:1000 000) has been used to infiltrate the excision area as well as donor site .
As the amount of blood loss during the operation is not known precisely, subsequent calculation depending on pre operative and post operative PCV readings, by using specific formula to calculate blood loss.
In 19 randomly selected patients, deep dermal and full-thickness burns covering 5-40% body surface were included.
Mean blood loss was 72 ml per each percent debrided and covered with skin graft, ranging from 15.6 ml for excision in the posterior trunk to 193 ml for excision of the upper limb with the hands involved.
The main advantages of this method are the reduction of blood loss, the prevention of uncontrollable profuse bleeding and decrease post operative blood transfusion.

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Keywords: Blood loss; Burns; Epinephrine; Skin grafting; Tangential excision; tumescent technique.

One of the most effective therapies in decreasing mortality from major thermal injury has been the early excision of the burn wound and its coverage [1]. Jackson and colleague pioneered excision and grafting, beginning in 1954, with burns of 3% total body surface area (TBSA) gradually increasing up to burns covering 30% TBSA[2,3] .

Early Excision produces massive blood loss' which often becomes a major factor that limits the area of excision, thus greatly influencing the survival and the morbidity of burned patients [4] .

Many methods of reducing intra-operative blood loss have been described: use of the tourniquet for excision on the extremities, application of a topical solution of epinephrine hydrochloride, infusion of I-desamino-8-D-arginine vasopressin, intravenous application of triglycyl-lysine-vasopressin, early burn wound excision (first 24 h post-burn) , electro coagulation of the larger capillaries and subeschar infiltration of vasoconstrictors such as ornithine 8 vasopressin (POR 8) or epinephrine hydrochloride [4].

Subeschar infiltration with epinephrine solution known as Tumescent technique , Tumescent is derived from the Latin tumidus meaning swollen [5]. The 'tumescent technique' utilizes subdermal or subeschar injection of a dilute epinephrine solution prior to debridement and grafting [6].

This technique is a method to limit the blood loss associated with tangential excision and auto grafting [7].

It has been described in the burn literature and has recently gained popularity with plastic surgeons by limiting blood loss during suction lipectomy [8].

Excision and grafting of the burn wound

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utilizing this approach, although frequently practiced , has not been well documented with regard to safety and efficacy [9].

Technical aspects of the procedure also vary from center to center. We report our initial experience with use of the 'tumescent technique' in burned patients with emphasis on quantifying efficacy, the safety of the procedure as well as intra-operative blood loss resulting from tangential excision of burned tissues. We also compare our study with other studies.

METHODS

This study reviews the intra operative blood loss In 19 randomly selected patients from adult age group, whereas female patients where 12(76.7%) and male patients where 7 (23.3%) patients with flame or scald burn injury with 5%-40% Total Body Surface Area (TBSA) burned who had at least one operation in Plastic and Burn hospital in Sulaimani city between Novembers 2009and December 2010.

Primary tangential excision (PTE) was performed as soon as possible in a period not exceeding 7 days after the admission (Figure 1).

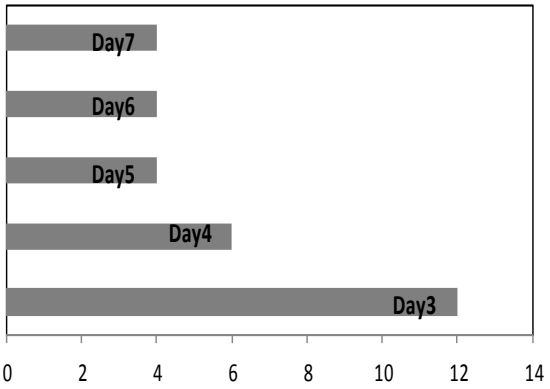

Figure 1.Number of surgical excision in relation to post burn days.

The TBSA excised not more than 20% in each session with different anatomical areas involved (Figure 2).

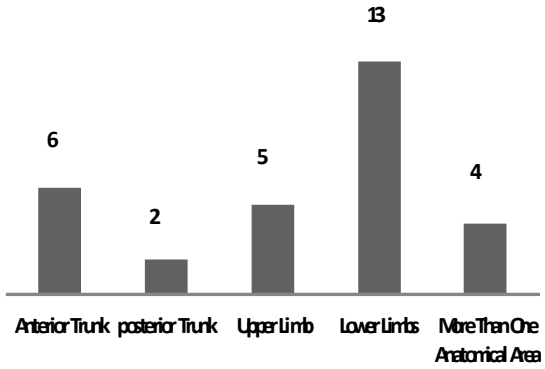

Figure 2. Anatomical areas involved in early excision and skin grafting in relation to number of burned patients

The technique of excision was preferably tangential; all the operations were performed utilizing the 'tumescent technique' to minimize blood loss.

In all patient general anesthesia were used, According to our strategy epinephrine is injected beneath the burn eschar and the donor site. The epinephrine solution used is composed of 1000 ml normal saline, with an added 1ml 1/1000 aqueous epinephrine hydrochloride (1mg epinephrine hydrochloride/1000ml solution, 1:1000 000).

Epinephrine solution was injected manually to the donor and subeschar sites, using syringes and needles.(Figures 3, 4, 5, 6, 7)

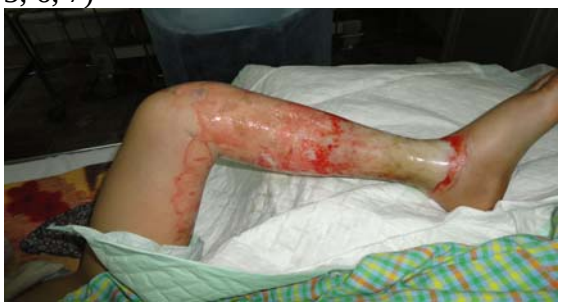

Figure 3. Third degree burn of leg.

Figure 4. Methylene blue applied to burn area before surgery.

Figure 5. Epinephrine injection to burn area and donor site.

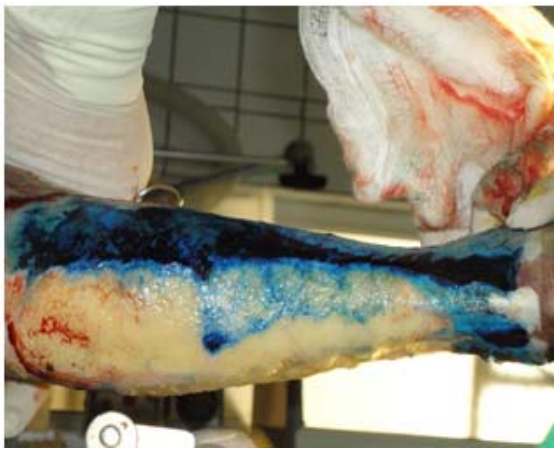

Figure 6. Excision of burn area without bleeding.

Figure 7. Blood less skin graft donor site.

The median volume of epinephrine solution injected is about I ml per kg of body weight per 1% debridement ,then excision started after 10-15 minutes after injection.

We used vital dye (methylene blue) applied 12 hours before operation in order to differentiate between viable and non viable tissue.

Blood loss intra operatively was estimated by clinical estimation, recording pre – operative Packed Cell Volume (PCV), and first and second day post operative PCV.

RESULTS

Total blood loss was determined by intra operative estimation and subsequent calculation depending on pre operative and post operative P.C.V. readings, by using this formula [10] to calculate blood loss:

$$\text{Blood loss (ml)} = \frac{\{(\text{preoperative RBCV}) + (\text{TX RBCV})\} - (\text{postoperative RBCV})}{\text{Post operative HCT} \times 0.01}$$

Where:

Preoperative RBCV (ml) = Body wt (kg) × 80 (ml\kg) (preoperative PVC)

Postoperative RBCV (ml) = Body wt (kg) X 80 (ml/kg) × [Postoperative Hct × (%) 0.01]

TX RBCV (ml) = Total mL whole blood administered during surgery × 0.3

Mean blood loss for each 1 percent body surface area debrided and covered was 71ml

The blood loss for each percent of body surface area debrided according to anatomical area as follows:

Ranging from 20.7 ml for excision of the lower limbs to193 ml for excision of both hands, with mean percent of total body surface area debrided in each session 12.57 % body surface area, and mean duration of operation 1 hour 39 min. (Table 1).

The mean of blood loss for excision each one percent of the total body surface area excised according to anatomical area as follows:

Posterior trunk (31.05ml)
Lower limb (51.28 ml)
For anterior trunk (71.22ml)
For upper limb (135.08ml)

For more than one anatomical area (except the hand) involved in on session (90.52 ml.)

P. Value estimated using SPSS (Statistical package foe social science) version 16 and it was significant operative Packed Cell Volume (PCV), and first and second day post operative PCV.

Table 1. Percentage debrided in each session, duration of operations and blood loss according to anatomical area excised in adult age group.

Case No.	Anatomical area debrided	Percentage debrided in each session	Duration of Operation In minutes	Use of methyline blue for depth estimation	Blood Loss In ml
Case No 1	Anterior trunk, Arms	20 %	105	Yes	86
Case No2	Anterior trunk	10 %	120	Yes	99.04
Case No3	Lower limb	14 %	90	Yes	69.8
Case No4	Anterior trunk , Arms	18%	105	Yes	92.8
Case No5	Lower limbs	4 %	60	Yes	43.3
Case No6	Lower limb	10 %	75	Yes	49.5
Case No7	Anterior trunk , upper limbs	20%	120	Yes	104.5
Case No8	Lower limbs	15 %	90	Yes	65.3
Case No9	Both hands and forearm	6 %	110	Yes	193
Case No10	Posterior trunk, upper limb	14 %	105	Yes	78.8
Case No11	Anterior trunk	9 %	75	Yes	16
Case No12	Lower limb	15 %	90	Yes	58.6
Case No13	Upper limbs and one hand	14 %	120	Yes	143
Case No14	Lower limbs	15 %	80	Yes	34.6
Case No15	Lower limbs	10 %	105	Yes	38.7
Case No16	Lower limb	10 %	60	Yes	20.7
Case No17	Lower limb	15 %	90	Yes	39.9
Case No18	Anterior trunk	10 %	90	Yes	69.3
Case No19	Lower limb	10 %	80	Yes	40
Mean		12.57 %	93		70.76
			P.Value* 0.0001		P.Value 0.0001

DISCUSSION

There are two problems in burn surgery: estimation of burn depth before and during the operation and estimation of blood loss [11].

The early excision of burn wounds may produce brisk, massive blood loss from the excised area. The intra-operative bleeding resulting from the excision of burned tissues often limits the area to be excised, with important effects on the morbidity and mortality of critically ill patients with thermal injuries [12].

After the excision of smaller burn wounds, patients often need transfusions following intra-operative blood loss [12].

The reduction of intra-operative blood loss following early excision of burn wounds therefore lessens morbidity and mortality in extensively burned patients. Also, in patients with minor burn wounds, transfusion may often be avoided [12].

That's why several techniques were used to reduce intra operative blood loss fallowing the early excision of the burn wound. In Sulaimani Burn and plastic Hospital adrenaline soaked gauze routinely used, but in the present study epinephrine solution was injected in the subeschar level at the burned site and subcutaneous level in the donor site in all body areas , and at any age group, except for those patient with history of cardio vascular diseases.

Although subcutaneous infiltration of epinephrine to reduce blood loss which often limits tangential excision of major burn wounds has been earlier described, and is known to be utilized by several burn centers. There is relatively little quantitative data on its efficacy and safety in the burn literature [13].

Prior studies by Vincent et al. have shown that saline infusion alone or the so called 'Pitkin Technique' is safe but does not significantly reduce the blood loss associated with donor site harvest [5].

Tumescent technique during the tangential excision and skin grafting of burn wounds can be utilized essentially on any part of the body.

After the injection of epinephrine solution, vasoconstriction become evident after few minutes, consequently bleeding after tangential excision of the burned tissue , which was carried out after ten minutes, and bleeding from donor site was significantly low by visual estimation (Figures 5,6) and by subsequent calculations.

These results compared to results of other studies (they did not use methylene blue) [14] (Table 2).

Table 2. Mean blood loss per each percent body surface area excised and covered in Sulaimani Burn & Plastic hospital compares to other studies results

	Mean blood loss
Sulaimani Burn & Plastic Hospital study	**71 mL per one percent body surface area excised and covered**
Early excision and skin graft without the use of adrenaline	**211 mL per one percent body surface area excised and covered**
Early excision and skin graft utilizing tumescent technique	**123 mL per one percent body surface area excised and covered.**

It is important to mention that with the use of adrenaline infiltration it is difficult to assess the adequacy of the depth of tangential excision due to change in the appearance of the burn wound due to vasoconstriction effect where the tissue look similar to those under tourniquet [12] .

In Sulaimani Burn & Plastic Hospital we use vital dye (methylene blue) 12 hours before the operation time which stain the necrotic tissue with blue color in order to assess burn depth and to excise full thickness injuries down to healthy tissue and this result in less time required for excision, and the excision of the dead tissue become easier and more precise. This resulted in less blood loss compared to other studies that depend tumescent technique without the use of methylene blue.

The other benefit of Infiltration that it can be used in areas where tourniquet is not possible to use, and for the donor sites is that simplifies the harvest of traditionally difficult areas such as the abdomen, scalp and allows the surgeon to take thinner grafts from conventional areas such as the thigh.

No adverse effects of the tumescent technique were encountered. There was no evidence of significant rebleeding as the epinephrine was metabolized, either in the operating room or later beneath grafts (Figures 7,8).

Although it is not quantitated, there was no subjective delay in the healing of donor sites or impairment of graft take.

Cardiovascular effects of the epinephrine were was too small to recognize during a single case. No arrhythmias were encountered.

CONCLUSIONS

- The subeschar infiltration of epinephrine solution is a simple and effective aid in primary tangential excision which controls profuse capillary bleeding

significantly and decrease the requirement for post operative transfusion in minor burns.

- It is very easy to learn and can be used even by surgical residents with limited surgical experience specially if used in conjunction with methylene blue to assess the depth of the burn wound .
- Not need expensive equipment in order to be infiltrated.
- 4-The use of adrenaline solution in extensive burns is associated with no or minimal transitory cardiovascular complications.
- Infiltration of donor sites simplifies the harvest of traditionally difficult areas such as the abdomen, scalp, and conventional areas such as the thigh.

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پوختە

بۆ کەمکردنەوەی لەدەستدانی خوێن بە لێدانی ئەپینەفرین لە ژێر سۆتەی سووتاوی و لابردنی سۆتە و متوربەکردنی پێست لە نەشتەرگەری سووتاویدا

کێشەیەکی تیغە لابردنی بریتی سووتاوی، بریتییە لە خوێنڕێژی : بۆ کەمکردنەوەی ئەم خوێنڕێژییە , گیراوەی 1000/1 ی ئەپینەفرین لە سورەمی خوێییدا, بەکارهێنرا .

ئەم گیراوەیە کرایە بە دەرزی کرایە ژێر سۆتەی سووتاوی و ئەو شوێنەی پێستەکەی لێوە دێنێن بۆ متوربەکردن.

لە بەر ئەوەی بە تەواوەتی هێندی خوێنڕێژییەکە لەم نەشتەرگەرییانەدا نەزانراوە , بارستەی خرۆکە سوورەکان پێش و پاش نەشتەرگەرییەکان ئەژمار کرا و بە هاوکێشەیەکی تایبەت ژمێردرا.

لە 19 نەخۆش کە بە هەڕەمەکی خرانە توێژینەکەوەو سووتاوی قولّی پێستییان هەبوو و لە (5–40٪) لەشی گرتبوونەوە, تێکڕای خوێنڕێژییەکە 72 مل بووو لە تانوپۆی 15.6 مل لە سووتاوی پشت و تەنگە و 193 مل لەوانەی پەلی سەرەوە و دەستییان سووتابوو.

سوودی سەرەکی ئەم رێگەچارەیە بریتییە لە کەمکردنەوەی خوێنڕێژی و لەدەستچوونی خوێن و کەمکردنەوەی پێویستی پێدانی خوێن بوو پاش نەشتەرگەرییەکان.

الخلاصة

زرق (ابينيفرين) تحت الخشارة في عملية الاستئصال المبكر والترقيع الجلدي لتقليل نسبة فقدان الدم في جراحة الحروق

احدى المشاكل الرئيسية لعملية الاستئصال العرضي لحالة الجرح الناتج من الحروق هو النزف الدموي، ولتقليل النزف يتم زرق مكان عملية الاستئصال بمحلول (ادرينالين) بنسبة (1:1000000)، ان كمية فقدان الدم اثناء العملية من الصعب قياسه بشكل دقيق ، لذلك معرفة او حساب الدم المفقود يعتمد على قياس (PCV) قبل العملية وبعد العملية ووفق معادلة خاصة لهذا الغرض .

تم اختيار (19) من مرضى الحروق بدرجة عميقة ونسبة حروقهم يتراوح بين (5–40)٪ وكان معدل الوسط الحسابي لفقدان الدم هو (72)مل لكل (1٪) من عملية الاستئصال والترقيع الجلدي وتراوح فقدان الدم لعملية الاستئصال في منطقة الظهر نسبة (15.6) مل لعملية الاستئصال في منطقة الاطراف العلوية وتراوحت نسبة فقدان الدم في منطقة الاطراف العلوية (193) مل.

أهم المزايا الرئيسية لهذه الطريقة بالنسبة للمريض هي، خفض نسبة فقدان الدم، والوقاية من النزف الشديد، وتقليل نسبة اعطاء الدم له.

A RANDOMIZED COMPARISON OF INTRAVAGINAL MISOPROSTOL FOR LABOR INDUCTION VERSUS EXPECTANT MANAGEMENTS IN PROLONGED PREGNANCY

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ABSTRACT

Background: Induction of labor for prolonged pregnancy is one of the commonest interventions in obstetrics.It is applied to 25% of pregnant women.
Objective: To compare elective induction of labor by intra vaginal misoprostol with expectant management (serial antenatal monitoring) in women at 41weeks and more.
Subjects and methods: This study involved 120 pregnant women with uncomplicated pregnancies, at gestational age of 41 weeks and more. The women were randomly assigned to undergo induction of labor by the intra-vaginal application of (50 microgram) misoprostol or to have serial antenatal monitoring, and waiting for spontaneous labor unless there was evidence of fetal or maternal compromise. The outcomes parameters were, maternal complication, mode of delivery, number of emergency cesarean sections performed for abnormal fetal heart rate, rate of neonatal morbidity and maternal satisfaction.
Results: The two groups were comparable in their maternal age, gestational age in weeks and number of pregnancies. There were lower rates in cesarean section 16.66 %versus53.44% , meconium stained liquor19.99%versus50% , APGAR Score < 7 in 5 minutes , neonatal intensive care unit admission 3.33%versus16%and rate of emergency cesarean section performed for fetal heart rate abnormalities 3.3%versus38.33% in induced versus the conservative group with higher rate of maternal satisfaction in induced group.
Conclusions: Induction of labor with misoprostol in post term pregnancy decreases maternal and neonatal morbidity compared to expectant management.

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Key words: misoprostol, post-term pregnancy, induction of labor, antenatal fetal monitoring.

Prolonged pregnancy is defined as a pregnancy that extends to 42weeks and beyond [1]. The reported frequency of post term pregnancy is approximately 5-10 percent in all pregnancies [2].

Studies have shown that a reduction in the number of pregnancies considered to be post-term is established when early ultrasound dating is performed [2] .

Antenatal surveillance with fetal kick counts, non stress testing, amniotic fluid index measurement, and biophysical profiles is used, although no data show that monitoring improves outcomes [2,3] .

Studies show a reduction in the rate of cesarean sections and possibly in neonatal mortality with a policy of routine labor induction at 41 weeks gestation[1,2] .

When post-term pregnancy exists the cause is usually unknown, but primiparity, previous post-term pregnancy and male gender are the most common identified risk factor for prolonged pregnancy[3,4] .Rarely it may be associated with sulfatase deficiency or fetal anencephaly [4]. Other most common cause of post-term pregnancy is inaccurate dating of gestational age which is more likely in women with irregular menses, those who seek prenatal care late in pregnancy and those with delayed ovulation (e.g. women who use oral contraceptive pills) [4] , or those who do not remember their LMP [5] .

There is an increased risk of fetal distress, low umbilical artery PH level at delivery, low 5 minute apgar scores and dysmaturity syndrome associated morbidity in post term birth[6,7] . The risk of meconium

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aspiration, asphyxia, bone fracture, peripheral nerve injury, pneumonia and septicaemia are also increased[7,8]. Umbilical artery compression from oligohydraminios, cephalopelvic disproportion, macrosomia and its complication which include prolonged labor, shoulder dystocia with the resultant increased risks of orthopedic and neurologic injury are also reported complications in prolonged pregnancy[9,10]

Oxytocin is the drug of choice for labor induction when cervix is favorable[11] . In a patient whose cervix is unfavorable, the use of prostaglandin analogs for cervical ripening markedly enhances the success of induction [11] .

The aim of this study is to compare elective induction of labor by intra vaginal misoprostol with expectant management (serial antenatal monitoring) in women at 41weeks and more to find which method is superior to improve fetal and maternal outcomes.

METHODS

This study was conducted as randomized trial, in maternity teaching hospital in Erbil city, Kurdistan region, Iraq. Study population included 120 pregnant women at 41 weeks or more of their gestation during period between first of November 2009 till first of April 2010.

Inclusion criteria: Intrauterine gestation as calculated from last menstrual period date confirmed by an ultrasound examination prior to 20 weeks to be 41weeks and more,single fetus with cephalic presentation with no history of prelabor rupture of membranes, no uterine contraction,Bishop score <6.

Exclusion criteria: Women with anomalous fetus,diabetes and other medical diseases (heart disease, pulmonary disease and etc),history of previous caesarean section or any type of uterine scar,history or evidence of disorders that represent contraindications to the use of misoprostol (e.g.: severe pulmonary disease, congenital or acquired heart diseases), prelabor rupture of membrane,non-reactive non stress test, patient with established labor and or (bishop score>6),known hypersensitivity to drugs, febrile condition, genital hemorrhage, twin pregnancy and patients refusal to participate in the study.

Demographic characteristics of each patient were assessed including gestational age, number of pregnancies, and history of previous post term pregnancy. Ultrasound scans for assessment of amniotic fluid volume (oligohydraminios was defined as amniotic fluid index less than 5cm or single deepest pocket less than 2cm), and clinical vaginal examinations to assess the cervix were also done.

Lastly biophysical profile was performed for each patient. The consent of the patients was taken for their enrollment in the study and was randomly selected for the type of treatment they received.

First group of the patients (groupA) were allocated to induction of labor (n=60women). They received an initial dose of 50 microgram of misoprostol placed intra vaginally in the posterior vaginal fornix, the dose was repeated every 6 hourly up to 4 doses , until the patient developed adequate uterine contraction, when patients developed established contraction pattern of>3 contractions in 10 minute misoprostol was stopped. Artificial rupture of membrane was performed for all patients when the fetal head was well applied to cervix and there was equal and more than 4cm dilatation. Continues external electronic fetal heart rate monitoring was used routinely. Patient in the active phase of labor (at least 4 cm dilatation), who subsequently had arrest of dilatation despite of adequate uterine contractions (no change in cervical dilatation >2 hour), received oxytocin augmentation.

If the cervix was still unfavorable, (bishop score <6, cervical dilatation <4 cm) after 4 doses of misoprostol, the procedure was

considered unsuccessful. In these cases the decision for further management was done either by augmentation with oxytocin or by abdominal delivery.

Second group of patients(groupB) underwent serial antenatal monitoring (fetal kicks, cardiotocography and assessment of amniotic fluid volume) every third day until spontaneous labor .When there was evidence of fetal or maternal compromise, labor was induced or cesarean section was performed.

The outcome parameter were the mode of delivery, maternal satisfaction, oxytocin use and post partum hemorrhage .Neonatal morbidity (meconium stain liquor,APGAR Score<7 in 5minute ,neonatal care unite admission, emergency cesarean section performed for abnormal fetal heart rate.) were assessed in both groups. Sex of fetus and birth weight were recorded .Supplementary medical information was obtained from the routine pediatric examination, and if admitted, from neonatal intensive care unit.

Ethical consideration: The verbal consent was taken from all the enrolled patients, all of them informed about the purpose and the procedure of the study. Permission from seniors on call who were managing the patients that included in the study.

Statistical analysis: The data was entered into computer using Microsoft excel version. Statistical package for social sciences (SPSSversion 16.0) was use for data analysis.Chi square test of association was used to compare proportions and frequencies.T –test was used to associate the difference between 2 means of variables.P –value of less than 0.05 was considered statistically significant.

RESULTS

Demographic characteristics of both groups were compared regarding maternal age, gravidity, parity and past history of post term pregnancy.

The mean maternal age for the induction group was (29.72±4.3) and the mean maternal age for the conservative group was (26.95±5.4). There was no statistical significance in maternal age between the two groups (p-value=0.482).

Twenty nine patients (48.33%) in the induction group were primigravida and 31 patients (51.66%) were multigravida compared with 26 primigravid patients (43.33%) and 34 multigravid patients (56.66%) in the conservative group.

Among the induction group, 10 patients (16.6%) had previous history of post term pregnancy compared with 16 patients (26%) who had previous history of post term pregnancy in monitoring group. A statistically significant difference did not exist between the two groups (p-value= 0.184) as shown in(Table 1).

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.*

Twenty five patients (41.66%) needed oxytocin augmentation in the induction group compared to 34 patients (56.7%) in the conservative group with (p -value = 0.103), which is statistically not significant as shown in(Table 2).

Sixteen patients achieved ripening of the cervix and started labor after a single dose of misoprostol, a further 30 required a second dose. Ten patients received three doses and four patients received four doses.

(Table 2) shows maternal outcomes in both groups: Fifty patients (83.33%) were delivered by vaginal and 10 patients (16.66%) by cesarean section in the induction group while 28 patients (46.66%) were delivered vaginally and 32 patients by cesarean section in the conservative group with a p value of 0.000, which is statistically significant.

With regards to the frequency of development of post partum hemorrhage, two patients (3.33%) in the induction group developed postpartum hemorrhage and three patients (5%) in the monitoring group with a p value of 0.64, which is statistically not significant.

Table 2 shows that 54 patients (90%) in the induction group were satisfied with their management, compared with 35 patients (58.33%) in the conservative group with a statistically significant p value of 0.000.

Regarding indication of cesarean section among the induction group, in five cases (8.33%),

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.*

cesarean section was conducted due to failure of induction, in two cases (3.33%), due to intra partum fetal distress and in three cases (5%), due to prolonged second stage of labor. In comparison, the conservative group had seven cases (11.66%) in which cesarean section was done due to failure of progress of labor, in 23 cases (38.33%), due to intra partum fetal distress and in two cases (3.33%), due to prolonged second stage with a statistically significant p value of 0.000 as shown in (Table 3).

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) shows fetal outcome in both groups:

The study's results showed that in the induction group 48 patients (80%) had clear liquor, 10 cases (16.66%) had thin meconium stain liquor and two cases (3.33%) thick meconium stain liquor, while in the conservative group 30 cases (50%) had clear liquor,18 cases (30%) had thin meconium stain liquor, and 12 cases

(20%) had thick meconium with a p value of 0.000, which is statistically significant.
APGAR score less than seven at five minutes was noticed in three newborns (5%) in induction group, and 10 newborns (16.7%) in the conservative group with a p value of 0.015, which is statistically significant.
It had been found that two newborns (3.33%) in the induction group admitted to the neonatal care unit while 16 newborns (26%) in the conservative group admitted to neonatal care unit with a p value of 0.000, which was statistically significant.
In regards to the emergency cesarean section performed for fetal distress, two cases (3.3%) in the induction group compared with 23 cases (38.33%) in the conservative group underwent emergency cesarean section for suspected fetal distress with a p value of 0.000, which is statistically significant.

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*

DISCUSSION

Prolonged pregnancy is recognized as a high-risk problem faced by obstetricians. Perinatal morbidity and mortality have increased significantly and, for that reason, most obstetric units offer routine induction of labor between 41 and 42 weeks of gestation to minimize the adverse perinatal risks. Debate continues regarding whether the policy of routine induction of labor is justified, particularly with an unfavorable cervix [12,13].
Regarding the rate of cesarean section, the result of the current study resembles the findings of the Canadian multicenter post term pregnancy trial group by Hannah ME et al [5] . The researchers randomized 3407 women with uncomplicated pregnancies at 41 weeks gestation or longer to the induction of labor or expectant management. Induction resulted in lower cesarean rate (21.2% versus 24% p=0.03).
The results of this study are also comparable to a systemic review with Meta analysis done by Sanches-Ramos L et al [14], which showed lower cesarean sections rate in those who underwent labor induction (20.1% versus 22%).
The study done by Gelison O et al[15] also showed a decreased rate of cesarean `section in the induction group (19.3% versus 22% with p=0.04).

However, this study's findings differ from the results of a randomized control trial done by Heimstad R et al[7] that included 504 women, who were randomly assigned with 254 in each group in their 289 days of pregnancy. They found no difference in the rate of cesarean section between the induction group and the conservative group (28 compared with 33, p=0.50).The lack of a difference in the cesarean rate of this study may be due to a lower prevalence of cesarean delivery in Norway (11-13%) than that described in the Canadian multicenter post term pregnancy trial (21-25%). Moreover, in our hospital the rate of caesarean delivery may be higher due to shortage of fetal blood sampling and advanced fetal monitoring techniques.

The history of previous post term pregnancy in the women included in this study is shown in (Table 1). These findings were supported by Nakling J et al's [8] study which reported 9.7% in a total of 130 cases of women who had a history of one or more post term pregnancies.

The rate of post partum hemorrhage, as shown in (Table 2), differed from the rate found in Duff C et al's [16] study. The mean blood loss in the induced group was greater by 24 ml which was a statistically significant difference. This difference may be due to the fact that it was an estimated blood loss rather than a measured blood loss.

Oxytocin use for augmentation of labor, as shown in (Table 2), disagreed with the studies done by Duff C et al [16] and Jeferson H et al [17], which found significant oxytocin augmentation in induction group.

The higher level of maternal satisfaction found in the induction group, as displayed in (Table 2), was consistent with the study conducted by Carrie Morantz et al [18] . This may be due to the higher rate of cesarean section, hospital visit for fetal monitoring and higher rate of fetal morbidity in the conservative group.

There was a higher rate of maternal satisfaction among the induction group.

There was a statistically significant difference between the induction group and the conservative group regarding fetal distress. In support of this, the studies conducted by British Columbia Reproductive Care Program [6] and Bradley A et al [19] found similar results.

Regarding meconium staining liquor, as shown in (Table 4), there was a statistically significant difference between the two groups, which was comparable with the results of the studies performed by Sanches-Ramos L et al[14], Crowley P(20), Gelison O et al[15] , Cuervo LG et al[21] and Bradley A et al[19] .

Regarding the APGAR score, as shown in (Table 4); it is similar to the findings of studies done by Nackling et al8, Sanches-Ramos L et al[14] and Duff C et al[16)].

In relation to the neonatal admission to NCU that is shown in (Table 4); it is comparable to the studies conducted by Hannah ME et al [5], Gulmezoglu AM et al[22] Sanchez-Ramos L et al[14] .

The effect of post term pregnancy on fetal weight was inconsistent with the result of the study done by British Columbia Reproductive Care Program [6] .

Regarding the effect of sex of fetus on post term pregnancy in this study, the results disagreed with the findings of both Hovi Mo et al.'s [23] and Crowley P's [5] studies, which showed that large proportions of fetuses were male in post term pregnancy.

The rate of emergency cesareanz section done for abnormal CTG as displayed in (Table 4) was in agreement with the study done by Sanches-Ramos L et al [14] .

CONCLUSION

Induction of labor with misoprostol in post term pregnancy decreases maternal and neonatal morbidity compared to conservative management. The rate of post partum hemorrhage and oxytocin augmentation was similar in both groups.

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پوختە

درێژ بوونەوەی ماوەی دوو گیانی بە کێشەیەکی سەرەکی تەندروستی دادەندرێت و دەبێت یەکسەر چارە بکرێت ئەگەر هاتوو لە کاتی دیاری کراوی خۆی تێپەڕی ، چونکە دەبێتە نیگەرانی هەردوو لا هەم دایك و هەم کۆرپەلەکەش

ئامانج لە توێژینەوە: ئامانج لە توێژینەوەی ئەم بابەتە دیاری کردنی بەراورد کارییە، لەسەر کاریگەری پاڵنەری هاندان لە رێگای (زێی ئافرەتەکەوە) (intra vaginal misoprostol) بە رێژەی 50 میکرۆگرام ، بەڵام لە ژێر چاودێری چڕ و ورد بۆ ئەو ئافرەتانەی تەمەنی سك پڕیان 41 هەفتەیە یان بۆ ماوەی پتریش کە لە رۆشنایی بەکار هێنانی سۆنەر دیاری دەکرێت و بابەتەکە دەکەوێتە ئاستی چارەسەر کردن دوای دەست نیشان کردنی دواکەوتنەکە.

شێوازی توێژینەوە: نمونەکان بە شێوازی هەڕەمەکی بۆ توێژینەوەکان و شیکردنەوەکان کۆ کراونەتەوە.

شوێن و ئامادەکاری توێژینەوە: ئەم توێژینەوە لە نەخۆشخانەی منداڵ بوون ئەنجامدرا لە ماوەی نێوان تشرینی دووەم تا نیسانی 2010.

لێکۆڵینەوەی بابتەکە و نەخۆشەکان: ئەم توێژینەوەیە 120 ژنی دوو گیانی لەخۆ گرتبوو کە ماوەی دووگیانیان گەیشتبووە 41 هەفتە و سەروتریش کە ئەوەش شێوازی کێشە تەندروستیەکەی لێکۆڵینەوەکەیە. بە شێوازێکی هەرەمەکی هەندێ لە نمونەکانی توێژینەوە هەڵبژێردران بۆ ئەوەی هاندانی پاڵنەری (زێ) یان بۆ بکرێت لە رۆشنایی چاودێری پزیشکی بەر لە ساتەکانی منداڵ بوون ، چونکە پێویستی بە ئامادە باشی و لێکۆڵینەوە هەیە نەوەك منداڵەکە یان دایکەکە توشی کێشەی تەندروستی ببنەوە.

شێوازی لێکۆڵینەوە و پشکنینی بەرایی پێش لە ساتەکانی منداڵ دانان بریتیبوو لە (الرکلات الجنینیە) پێکوتە کاری منداڵەکە ، لە منداڵدان پشکنینی تەنگەتاونەبوون non-stress و رێژەی ئاوی دەور و بەری منداڵەکە لەگەڵ وێڵداش.

دەرەنجامی کۆتایی: هەردوو گروپەکان بەراوردیان بۆ کرا لە دەروازەی: تەمەن و تەمەنی دوو گیانی بوون بە هەفتە و چەند جار سکی کردووە و هتد ..

رێژەی پارامیترەکانی خوارەوە کەمتر بوون بە بەراورد لەگەڵ رێژەی ئەم ئافرەتانەی بە نەشتەرگەری قەیسەری دەربازیان بوو (بە رێژەی 10 بۆ 32، p=0.00) ، نزم بوونەوە تێکچوونی رێژەی شلەی ناو وێڵداش (12 بە بەراورد بۆ 30، p=0.00) ، رێژەی APGAR < 7 لە پێنج خولەکدا (3 بە بەراورد بۆ 10 ، p=0.015) رێژەی وەرگیراوی NCU (2 بە بەراورد 16) هەروەها رێژەی نەشتەرگەری فریاکەوتنی قەیسەری بۆ ئەو کۆرپەلانەی کێشەی دڵیان هەبوو (2 بە بەراورد 23 ، p=0.00).

پیادە کردنی شێوازی هاندان و پاڵنەر لە رێگای (زێ) بەر لە ساتەکانی ژان گرتن و منداڵ دانان دەبێتە هۆکاری ئاسانی بۆ دایك و زووتر رزگار بوونی لە کاتی منداڵ داناندا. بەڵام رێژەی بینینی سەراو و خوێن پژان و کێشی کۆرپە و زێدە رێژەی ئۆکسی تۆسین لە هەردوو گروپەکاندا بە یەکسان خۆیان نیشاندا. بەڵام رێژەی سەلامەتی دایکەکان لە گروپی هاندەری پاڵنەر پتر بەرجەستە بوو.

الخلاصة

دراسة مقارنة بين استعمال الميزوبريستول عن طريق المهبل لتوسيع عنق الرحم و بدء الولادة و بين المتابعة التحفظية للحوامل بعد الاسبوع الواحد والاربعون من الحمل

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.

الهدف من الدراسة: هدف الدراسة هو المقارنة بين الكفاءة و المقبولية لعقار الميزوبرستول عن طريق المبهل لبدء الولادة مع متابعة الحمل فى اسبوع الواحد والاربعون من الحمل و الذى تم قياسة بالموجات فوق الصوتية (جهاز السونار) فى وقت مبكر من الحمل.

نوع الدراسة: اجريت هذة الدراسة فى مستشفى الولادة التعليمى فى اربيل فى الفترة مابين تشرين الثانى2009 لغاية نيسان 2010 وهي دراسة تقدمية عشوائية متوازية المجاميع من نساء حوامل. تضمنت هذة العينة 120 امراة حامل لغاية 41 اسبوع من الحمل مع حالات الحمل غير معقدة و قد تم قياس فترة الحمل عن طريق جهاز الموجات فوق الصوتية (السونار) بداية الحمل.

قسَمّت هذة العينة عشوائية الى مجموعتين اشتملت كل مجموعة على 60 امراة حامل المجموعة الاولى اعطيت عقار الميزوبروستول عن طريق المهبل و فق البروتوكول الاتى :50 ميكروغرام كل 4 ساعات حتى بدء الولادة و المجموعة الثانية تمت متابعة الحمل لحين بدء الولادة الطبيعية او حصول خلل ما فى حالة الطفل او الام , فى هذة الحالة تم اجراء بدء الولادة او اجراء العملية القيصرية. و تمت متابعة الحمل عن طريق حساب حركة الطفل,NST, و تقيم لحجم السائل الامنيوسى بجهاز السونار.

العوامل الرئيسية لهذه الدراسة: نسبة الخطورة على الام (maternal morbidity)، الولادة بعملية قيصرية او الولادة الطبيعية، نسبة الخطورة على الطفل (morbidity)، ارتياح الام للعلاج.

نتائج الدراسة: اظهرت الدراسة ان الاستجابة لبدء الولادة فى الاسبوع الواحد والاربعين من الحمل بعقار الميزوبروستول ادى الى ادنى نسبة فى العملية القيصرية (16٪ مقارنة ب 53٪), تلوين السائل الامينى (20٪ مقارنة ب 50٪), نقاط APGAR اقل من 7(5٪ مقارنة ب 16٪), نسبة احالة الطفل الى وحدات حديث الولادة (الخدج) (3.3٪ مقارنة ب 26٪), و معدل حالات عمليات طارئة بسبب تغير فى معدل ضربات قلب الجنين (3.3٪ مقارنة ب 38٪) . اعلى معدل اقتناع الام للعلاج كانت فى مجموعة العلاج بميزوبروستول (90٪ مقارنة ب 58٪)، لوحظ عدم وجود اى اختلاف احصائى بالنسبة الى حدوث النزف بعد الولادة , استعمال مادة أوكسيتوسين و وزن الطفل.

حصيلة الدراسة: اثبتت الدراسة ان استعمال عقار الميزوبروستول عن طريق المهبل لبدء الولادة في الاسبوع الواحد والاربعين من الحمل له فعالية كبيرة فى تقليل نسبة العمليات القيصرية الباردة والطارئة,ونسبة تعب الاجنة والاطفال حديثي الولادة

INCIDENCE OF *TOXOPLASMA GONDII* IN PLACENTA OF ABORTED FETUS

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ABSTRACT

Toxoplasma gondii infections can cause serious complications in pregnant women, leading to miscarriage, stillbirth, and birth defects. Diagnosis of *T. gondii* by Polymerase chain reaction (PCR) in human placenta of aborted fetus targeted B1 gene has been conducted. In this study, a total of 79 blood samples and the placentas of aborted fetus were collected from woman who had abortion, at the Maternity Hospital of Erbil city, Kurdistan region, northern of Iraq. The blood samples were examined by using a latex agglutination test (LAT). QIAamp DNA Mini Kit method was used for genomic DNA extraction from placenta. Polymerase chain reaction was applied on all samples and 115 bp fragment of B1 gene amplified. Polymerase chain reaction products were run in 2.5% agarose gel and visualized by UV light after ethidium bromide staining. Out of 79 serum samples, 75 (95%) cases were positive for anti *T. gondii* antibodies by LAT. From 79 placenta samples, nine (11.38%) were positive by PCR. Seroprevalence of *T. gondii* infection was very high among women who had abortion and a strong relation was observed between toxoplasmosis and abortion using PCR method.

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Key words: *Toxoplasma gondii*, Placenta, *B1* gene, PCR, Abortion.

Toxoplasma gondii infects warm-blooded vertebrates, including human. The parasite is distributed widely in the human population, and is estimated to affect more than a billion individuals worldwide [1]. Human infections are primarily caused by ingesting uncooked meat containing infective stages of *T. gondi*i or by ingesting food or water contaminated with sporulated oocysts shed in the faeces of infected cats [2]. Infection acquired during pregnancy may cause severe damage to the fetus. Most primary infections in adults are asymptomatic but in some patients lymphadenophathy or ocular toxoplasmosis can occur. In immunocompromised patients, reactivation of latent infection can cause life-threatening encephalitis [3]. Congenital toxoplasmosis may cause abortion, neonatal death or fetal abnormalities [4].
The laboratory diagnosis relies till now upon serology, this may be unreliable in those who are immunodeficient, as in the fetus isolation of the parasite by animal inoculation or cell culture, which is time consuming, demonstration of the parasite genome by PCR technique, a promising method due to its reported good sensitivity and high specificity [5,6] For this purpose PCR with placental tissues, has been proved of value [7]. The PCR targets described for *T. gondii*, the 35-copy number B1 gene is the most widely used [8].

The aim of this study was to investigate the occurrence of toxoplasmosis in aborted fetus in Erbil city by using PCR and Latex agglutination test (LAT).

METHODS

In 2009, a total of 79 blood samples and the placentas of aborted fetus were collected from woman who had abortion, from Minor Surgery Unit at the Maternity

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Hospital in Erbil city, Kurdistan region, North of Iraq. Mother age, fetus age and number of abortion were recorded.

Serological test

Detection of anti *T. gondii* antibodies in blood of mother by Latex agglutination test (LAT) has been used for diagnoses (Biokit, S.A) according to manufacturer's instruction.

Placenta preparation

Fresh placenta was homogenized in saline in a mortar (4000 rpm for 1 minute). The homogenate from each placenta was filtered through 2 layers of gauze. The filtrate was centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded and the pellet kept. The pellet was suspended in 0.5 ml sterile phosphate buffered saline (PBS) stored at -20 ºC for genomic DNA extraction (Bretagne et al., 993).

DNA extraction

Genomic DNA was extracted from placental tissues using the tissue protocol (Qiagen) according to the manufacturer's instructions. DNA was eluted in 100 µl elution buffer.

Target and primers

The target gene for amplification was the 35 fold repetitive B1 gene of T. gondii. B1 primers (B1F; AACGGGCGAGTAGCACCTGAGGAGA and B1R; TGGGTCTACGTCGATGGCATGACAAC) were synthesized at CyberGene, established by Bretagne et al[3] . The complete sequence of B1 gene is 2214 bp and the accession number in Gene Bank is AF179871. In the present study, the expected fragment size of PCR product was 115 bp.

PCR amplification

The optimized PCR conditions were based according to Bretagne et al [9] and Chabbert et al. [10] with some modifications : 1X buffer (10 mM Tris-HCl pH 8.8 at 25 °C), 50 mM KCl, 0.08% Nonidet P40), 0.6 mg bovine serum albumin (BSA), dNTPs at a concentration of 200 µM each, 2 mM MgCl2, 20 pmol of both primers (forward and reverse primer), and 1 U of Taq DNA polymerase, for a total volume of 50 µl and 5 µl of template DNA. The reaction mixtures were cycled in a thermal cycler (PxE Thermo) by using the following condition: denatured 3 minutes at 94 °C, followed by 40 cycles of 30 seconds at 94 °C ; 30 seconds at 59 °C; 30 seconds at 72 °C and finally extended 10 minutes at 72 °C.

Deoxyribonucleic acid extract of cultured *T. gondii* (Genekam) was used as positive control (PC). Placentas of seronegative mothers were used as a negative control sample (NCS). Also, ddH2O was used as a negative control (NC). The PCR products were visualized under UV light after electrophoresis in a 2.5% agarose gel on 70V for 30min. according to Sambrook and Russell [11]. All gels presenting band of the expected size (115 bp) were subjected to positive sample. If there is no band on gels, it was considered as a negative sample.

RESULTS

Latex Agglutination Test

Out of 79 serum samples of women who had abortion in Erbil city, 75 (95%) cases were positive for anti *T. gondii* antibodies and 4 (5%) cases were negative. (Table 1) shows the occurrence of anti *T. gondii* antibodies titers in the seropositive samples. The high titer was 1:320IU with seropositively of 65.3%, and the low titer was in 1:20 with seropositively of 1.3%.

Table 1. The occurrence of anti *T. gondii* antibodies titers in the seropositive samples.

Titers / IU	1:10	1:20	1:40	1:80	1:160	1:320
No. (%)	2 (2.6%)	1 (1.3%)	4 (5.3%)	3 (4%)	16 (21.3%)	49 (65.3%)

Placenta samples

T. gondii DNA were successfully amplified from nine placenta samples which constitute 11.39% of 79 samples (Table 2 and Figure 1). While there was no amplification of *T. gondii* DNA from other 70 samples which correspond 88.6%.

Table 2. Positive placenta samples for *T. gondii* by PCR.

No.	Mother age\year	Fetus age\week	Abortion times	Antibody titer/IU
1	32	6	First	1:320
2	30	12	First	1:320
3	35	8	Second	1:320
4	30	9	Fourth	1:320
5	29	6	First	1:320
6	25	14	First	1:320
7	37	10	First	1:320
8	37	5	First	1:320
9	35	8	First	1:320

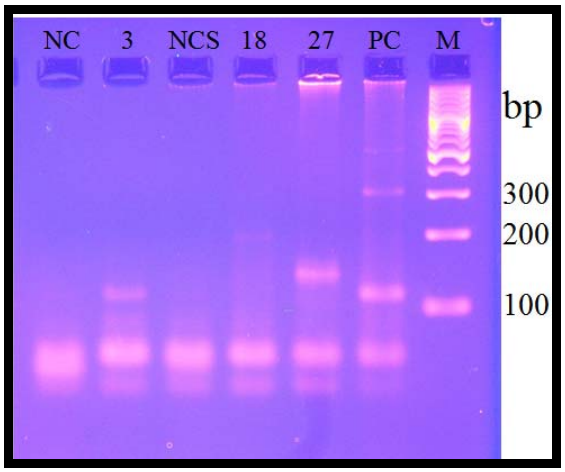

Figure 1. PCR results of placentas. Detection of amplified products after 2.5% gel electrophoresis and ethidium bromide staining, ~115 bp fragment corresponds to *T. gondii* amplified DNA. Lane M, 100 bp marker DNA; lane NC, Negative control; lane NCS, Negative control sample; lane PC, Positive control; lanes 3, 18, and 27 assayed placenta samples.

DISCUSSION

The detection of *T. gondii* DNA by PCR amplification has some advantages over conventional diagnostic methods. Molecular tests that could detect the presence of parasites would be extreme application. A positive serological result is only indicative of infection, whereas direct detection of *T. gondii* in placenta or other clinical samples categorically confirms the parasite presence leading to the diagnosis of primary, reactivated or chronic toxoplasmosis [12]

The results of the present study showed that the seroprevalence of anti *T. gondii* antibody in mother of aborted fetus in Erbil city were very high (95%) by using LAT. But in the previous studies conducted in Erbil city it was low. Studies such as Bakir [11] found 71.5%, Akreyi [14] recorded 84% and Hamad [15] reported 54.4% by using LAT. In general, Rey and Ramalho [16] mentioned that seroprevalence of *anti T. gondii* antibody in different regions in the world is ranged between 15-100%. The difference in the seroprevalence of toxoplasmosis in different studies may be due to the difference in the serological tests and or the sample size as well as the condition of each setting area.

Despite the introduction of molecular techniques, the diagnosis of congenital toxoplasmosis poses distinct problems. Prenatal diagnosis by PCR amplification performed on amniotic fluid is a possibility when the mother experiences symptomatic toxoplasmosis during pregnancy. Diagnosis in the antenatal period is advantageous, giving an opportunity to introduce early treatment [17].

Successful identification of *T. gondii* B1 gene was observed in 9 placenta samples (11.39%) out of 79 cases, suggesting acute *T. gondii* infection with risk transmission to fetus and subsequent caused abortion.

In the present study PCR technique was used for detection of *T. gondii* DNA in the placenta. The method, based on the 35-fold repeated B1 gene, is sensitive [it allows to detect as few as 0.1 pg parasite DNA (about one parasite) in the presence of human genomic DNA] and specific. The B1 gene is apparently repeated 70 fold in the *T. gondii* genome by QC-PCR [18]. This copy number is slightly different from the previously reported one (35 fold) [8].

The incidence of toxoplasmosis results from exposure to different factors for example, in Erbil city, high prevalence of toxoplasmosis were recorded because of wrong diagnosis, the deficient of health conscious, inadequate of enough treatment, little epidemiological information and asymptomatic of the disease. The present results may be explained by the high exposure chances of population to the parasite throughout their life, absence of personal hygiene, food habits, low socioeconomic status and the close contact with oocyst in feces of infected cats which contaminate soils. *T. gondii* infection was positively associated with eating raw meat in pregnant women [2]. The cooking temperature of meat is an important issue in the infection of *T. gondii*. Thorough cooking it is always preferred in Erbil city, Iraq.

Because of lack of molecular diagnose in Iraq, there is a little knowledge about the rate of infection with *T. gondii*, maternofetal transmission and abortion caused by *toxoplasmosis*. It is important when seroconversion happens to pregnant women, *T. gondii* detect in amniotic fluid by PCR. This technology should be restricted to specialized centers [19]. Because parents request termination of pregnancy after a positive prenatal diagnosis, it seems advisable not to depend solely on the PCR technology for such decisions.

In conclusion, seroprevalence of *T. gondii* infection was very high among women who had abortion and a strong relation was observed between toxoplasmosis and abortion using PCR technology among patients in Erbil city, Iraq.

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پوخته

ڕێژەی تووش بوون بە مشەخۆر *Toxoplasma gondii* لە وێڵاشی منالّی لە بار چوو

تووش بوون بە مشەخۆر *Toxoplasma gondii* دەبێتە هۆی زۆر لە نەخۆشی ترسناك لە ئافرەتی دوو گیاندا كە دەبێتە هۆی لەبار چوونی زووی منالّ یان مردنی منداڵ لەناو منداڵدان یان نەخوشی زكماك. مشەخۆر *T. gondii* لە وێڵاشی منالّی لە بار چوو بینرایەوە بە بەكارهێنانی ئامێری PCR بە چەند هێندەكردنی بوهێڵی B1. 79 نموونە لە خوێن و وێڵاش پشكێنران كە لە ئافرەتی منالّ لە بارچوو لە نەخۆشخانەی ناوەندی منالّ بوون لە شاری هەولێر/ هەرێمی كوردستان– باكووری عیراق كۆكرابوونەوە. نموونەی خوێنەكان بە بەكارهێنانی تاقی كردنەوەی مەیینی خوێن (LAT). هەروەها كەتەلۆكی كۆمپانیای QIAamp بەكارهێنرا بۆ پاڵاوتنی ماددەی بۆماوەیی لە وێڵاشەكان. PCR بۆ گشت نموونەكان بەكارهێنراو پارچەیەكی 115 bp لە بوهێڵی B1 چەند هێندەكرا. دەست كەوتوەكانی PCR لەسەر جیلی ئاگاری 2.5٪ بەڕێ كرا و دەرخسترا بە بۆیەكردن بە بڕۆمیدی ئیسیدیۆم. لە كۆی 79 نموونەی زەردەوی خوێن 75 نموونە (95٪) ئەرێی نیشاندا بۆ دژە دژەتەنی *T. gondii*. لە كۆی 79 وێڵاش تەنها 9 وێڵاش (11.38٪) ئەرێی بۆ PCR بیشاندا. ڕێژەی تووشبوونی زەردەی خوێن لە نێوان ئافرەتانی منالّ لەبار چوو زۆر بەرزە هەروەها پەیوەندیەكی بەهێز لە نێوان تووش بوون بە T. gondii و لەبارچوون بە بەكارهێنانی PCR بینرا.

الخلاصة

معدل حدوث الطفيلي*TOXOPLASMA GONDII* في مشيمة الأجنة المجهضة

ان الاصابة بالطفيلي *Toxoplasma gondii* يسبب مضاعافات خطيرة لدى النساء الحاملات مؤديا الى الاجهاض المبكر او وفاة الجنين داخل الرحم او تشوهات خلقية. تم تشخيص *T. gondii* من مشيمة الاجنة المجهضة بواسطة استخدام جهاز PCR عن طريق تضخيم المورثة B1. حيث تم دراسة 79 عينة من الدم والمشيمة في الدراسة الحالية، والتي جمعت من النساء المجهضات من مستشفى الولادة العامة في اربيل/ اقليم كوردستان– شمال العراق. درست عينات الدم باستخدام اختبار تجلط الدم (LAT). كما واستخدم كاتالوك شركة QIAamp لاستخلاص المادة الوراثية من المشايم. استخدمت PCR للنماذج كلها وتم تضخيم قطعة 115 bp من الجين B1. تم تهجير منتجات PCR على جلي الاكار 2.5% و اظهرت للعيان بالاشعة الفوق البنفسجية بعد التصبيغ باستخدام بروميد الاثيديوم. من مجموع 79 نموذجا من نماذج المصل 75 (95٪) نموذج اظهرت نتائج ايجابية لمضادات الاجسام المضادة للـ *T. gondii*. من مجموع 79 مشيمة تسعة فقط (11.38٪) اظهرت نتائج ايجابية للـ PCR بسبب الاصابة المصلية بالابتدائي *T.* gondii بين النساء المجهضات عالية جدا ولوحظ ايضا علاقة قوية بين الاصابة بالـ *T. gondii* والاجهاض باستخدام الـ PCR.

IMPACT OF HEALTH EDUCATIONAL PROGRAM UPON KNOWLEDGE AND PRACTICES OF CAREGIVERS HAVING ACUTE LEUKEMIC ADOLESCENT PATIENTS

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ABSTRACT

Background: Acute leukemia is a malignant hematologic disorder characterized by a proliferation of immature WBC that infiltrates the BM, peripheral blood, and other organs. Home care is the fastest growing component of the health care, and caregivers provide and construct important emotional and physical care for a person with cancer.
Objectives: To improve knowledge and practice of caregivers having adolescent leukemic patients.
Methods: A quasi experimental study was carried out in Nanakali Hospital for Blood Disease / Erbil city from the period of 1st Nov. 2010 to 1st Nov. 2011 . Seventy caregivers were participated in the study (35 controls and 35 study group).
Results: Most of the caregivers were mothers, their mean age was (41.7±9.176 and 40 ± 8.15) years old, the highest percentage were illiterate, coming from rural areas, living with low socioeconomic status (SES), the mean age of adolescent leukemic patients were (14.94 ± 2.950, 14.83±3.535) years old, most of the adolescent leukemic were males, and having type Acute lymphocytic leukemia (ALL) , in the control and study groups respectively. The result indicates that there are highly significant differences between pre-test and post-tests (1 and 2) related to caregivers' knowledge and practices at p-value (.001, 028, 003 and .012) in the study groups respectively.
Conclusion: The study reveals to improving the knowledge and practices of caregivers having adolescent leukemic patient related to study groups after implementation of health educational program.

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Key words: knowledge, Practices, Caregivers, Leukemic Adolescent.

Acute leukemia results from uncontrolled proliferation of immature lymphocytes; it is responsible for at least one third of deaths in children and teenagers[1] . Approximately 3,600 children were diagnosed with acute leukemia in the United States in 2000 [2]. Depending on statistics obtained from Nanakali Hospital for blood diseases (2011), more 3900 cancer patients have been diagnosed and the age of one third of the patients has been less 20 years [3] .

Adolescents with acute leukemia represent a major challenge to healthcare professionals they have a serious illness. This period of life is an important period of growth and development that involves significant psychological, social, and maturational adjustments as adolescents move toward adulthood [4,5.]

Most adolescents experience unpleasant physical side-effects, behavioral and emotional problems and the risk of late effects including reduced linear growth, compromised endocrine sensory functions, and damage to the cardiac and reproductive system[6] .

At home a caregiver is the main provider of physical and emotional support for the patient. Caregivers are mostly the patient's

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spouse, partner or closest relatives, although care giving may lead to physical and psychological symptoms such as depression, anxious. Females are more tuned to the caring function at home [7]. More than half of all women will provide care for an ill or disabled person at some point in their lives and most of the caregivers are children's mothers [8] . Caregivers provide and construct important emotional and physical care for a person with cancer [9] . The home care is a good choice for many children who have cancer. Home caregivers can provide drugs, pain killer, equipment such as hospital beds or wheelchairs, proper nutrition, physical therapy, and many complicated nursing and medical care procedures [10] . Nurses should understand the stress of the parents. In addition, they should make relationships and provide psychosocial support to these parents so that they can establish their situation and take good care of their children and improve their quality of life (QoL) [11] .
Health education is primarily at preventative level and aims to increase knowledge thus enabling informed choice in addressing issues that affect health and well-being[(12)] . Survivorship educational programs may empower childhood cancer survivors towards healthier behaviors and lifestyles as well as regular participation in treatment care, which, in turn, may prevent or reduce risks of late, effects [11] .

METHODS

This is a quasi-experimental (Quantitative) study, carried out in Nanakali Hospital for Blood Diseases, from the period 1st of Oct. / 2010 to 10th of Oct. / 2011. Official permission was obtained from Erbil Health Directorate and Nanakali Hospital for Blood Diseases. Data collection is done by the researcher, who kept the confidentiality and anonymity of the data. The purpose of the study was explained to all participants; a verbal agreement was obtained from participants in the study. A non-probability (purposive) sample of (70) of caregivers was prepared. Thirty five (35) as a study group and another (35) as (a control group). Ten (10) of caregivers were dropped out from the groups. The educational health program concentrated and was implemented through six sessions; each session was designed and scheduled for approximately (60) minutes, one session each day. A questionnaire was concerning caregivers' knowledge. The question consists of (18) items related to the knowledge questions. The items are rated between (2) for Yes, and (1) for No, and the level of scale were scored as: (18) scores related to low knowledge and (36) scores related to the high knowledge, for assessment of caregiver's practices. It consists of (22) items rated between (2) for Yes, and (1) for No, and the levels of scale were scored as: (22) scores related to low practices and (44) scores related to good practices. A pilot study was conducted on (12) caregivers, who were randomly selected. Validity of the study instrument and program was determined initially through the panel of (14) experts. Correlation coefficient of the study was to determine the reliability of the study instrument. Data was collected by using questionnaire format and filled out by the investigator; Data were analyzed using the statistical package for social science (SPSS, version 17). (Basic Descriptive, t-test independent and one way analysis of variance) were applied. The P-value equal or less than 0.05 was considered as statistically significant.

RESULTS

Most of the caregivers (65.7%, 23 and 71.4%, 25) were mothers (Table1a). Their mean age was (41.7±9.176 and 40 ± 8.15) years old (Table 1b), the highest percentages were illiterate (Table1c), coming from rural areas (Table1d), and living with low SES (Table 1e). The mean

age of adolescent leukemic patients were (14.94 ± 2.950, 14.83±3.535) years old (Table 2a), most of them were males (Table 2b), and having type ALL, (Table 2c) in the control and study groups respectively. The result indicated that there is no significant difference between control and study group before implementation of health educational program (Table 3), and there is no significant difference between pre and post-test (1 and 2) related to control group (Table 4, and 6). But after implementation of health educational program , the present study shows that there are highly significant differences between pre and post-tests (1 and 2) related to caregivers' knowledge and practices at p-value (.001, 028, 003 and .012) (Table 5), in study groups respectively , and shows that there is a highly significant difference between pre and post-tests (1 and 2) relative to caregivers' knowledge and practices in the study group at p-value (.011 and 005) (Table 7), in the study groups respectively, and there is a highly significant difference between post-tests (1 and 2) in the control and study groups related to caregivers' knowledge at p-value (.007 and .015) (Table 8), in the study groups respectively., and shows that there is a highly significant difference between post-tests (1 and 2) in the control and study groups related to caregivers' practices at p-value (.006 and .018) (Table 9), in the study groups respectively.

Table 1. Demographic Characteristics of Caregivers.

Variables	Controlgroup n=35 No. (%)	*Study group n=35 No. (%)*
a. The person responsible for caring		
Mother	**23 (65.7)**	***25 (71.4)***
Father	**4 (11.4)**	***3 (8.6)***
Both parents	**3 (8.6)**	***2(5.7)***
Sister	**2(5.7)**	***00 (00)***
Brother	**2 (5.7)**	***3 (8.6)***
Aunt	**1(2.9)**	***2 (5.7)***
b. Age of caregivers/years		
19-25	**3 (8.6)**	***2 (5.7)***
26-32	**1(2.9)**	***5 (14.3)***
33-39	**13(37.1)**	***4 (11.4)***
40 and above	**18(51.4)**	***24 (68.6)***
Mean age ± SD	**41.7±9.176**	***40 ± 8.15***
c. Level of education		
Illiterate	**17 (48.6)**	***15 (42.9)***
Can read and write	**10 (28.6)**	***12 (34.3)***
Primary school graduate	**3 (8.6)**	***4 (11.4)***
Secondary school graduate	**00 (00)**	***1 (2.9)***
Preparatory school graduate	**3 (8.6)**	***1 (2.9)***
Institute and collage graduate	**2(5.6)**	***2 (5.6)***
d. Residency areas		
Urban	**10 (28.6)**	***14(40)***
Rural	**25 (71.4)**	***21(60)***
e. Socio-economic status		
Low	**25 (71.4)**	***26 (74.3)***
Middle	**10 (28.6)**	***7 (20)***
High	**00 (00)**	***2 (5.7)***

Table 2. Demographic Characteristics of Acute Leukemic Adolescent.

Variables	Control group n=35 No. (%)	Study group n=35 No. (%)
a. Adolescent's period		
Preadolescent	13 (37.14)	11(31.43)
Middle adolescent	11 (31.43)	10 (28.57)
Post (late) adolescent	11 (31.43)	14 (40)
Mean age ± SD	14.94 ± 2.950	14.83±3.535
b. Gender of adolescent		
Male	22 (62.86)	24 (68.57)
Female	13 (37.14)	11 (31.43)
c. Type of Acute leukemia		
ALL (Acute Lymphocytic Leukemia)	25(71.44)	27 (77.1)
AML (Acute Myloid Leukemia	10 (28.56)	8 (22.9)

Table 3. The comparative differences between caregivers' knowledge and practices with the control and study groups at pre-tests.

Groups	Control group Pre-test		Study group Pre-test		t.	P-value
Variables	MS	±SD	MS	±SD		
Knowledge	22.89	3.513	22.37	4.159	-0.559	.578
Practice	26.66	3.629	26.63	3.135	-0.35	.972

Table 4. The comparative differences between caregivers' knowledge and practices with the control groups at pre and post-tests (1 and 2).

Group	Control groups									
	Pre – test		Post-test 1		P-	Pre – test		Post-test 2		P-
Variables	MS	t.	MS	t.	value	MS	t.	MS	t.	value
Knowledge	22.89	-0.810	23.60	-.809	.421	22.89	-0.810	23.67-	-.882	.381
Practice	23.60	-1.261	27.83	-1.254	.422	23.60	-1.261	27.90	-1.31	.191

Table 5: The comparative differences between caregivers' knowledge and practices with the study groups at pre and post tests (1 and 2).

Group	Study groups									
	Pre – test		Post-test – 1		P-	Pre – test		Post-test -2		P-
Variables	MS	t.	MS	t.	value	MS	t.	MS	t.	value
Knowledge	22.37	-10.354	33.17	-10.341	.001	22.37	-10.354	32.70	-9.077	.003
Practices	26.63	-11.149	40.03	-10.638	.028	26.63	-11.149	38.13	-8.882	.012

Table 6. One way of variance (ANOVA) between pre and post-tests (1 and 2) in the control group.

Control groups Variables		Sum of Squares	df.	MS	f.obs.	P-value
Knowledge	Between Groups	8.242	1	8.242	.657	.421
	Within Groups	790.743	63	12.551		
	Total	798.985	64	20.793		
Practices	Between Groups	22.348	1	22.348	1.589	.212
	Within Groups	886.052	63	14.064		
	Total	908.400	64	36.412		

Table 7. One way analysis of variance (ANOVA) between pre and post-tests (1 and 2) in the study group.

Study groups / Variables		Sum of Squares	df.	MS	f.obs.	P-value
Knowledge	Between Groups	1610.196	2	805.098	70.595	.011
	Within Groups	1037.804	91	11.404		
	Total	2648.000	93	816.502		
Practices	Between Groups	44585.886	2	22292.943	446.871	.005
	Within Groups	4539.694	91	49.887		
	Total	49125.580	93	22342.830		

Table 8. A comparison between mothers knowledge with the study and control groups at post-tests (1 and 2).

Groups	Post-test 1			Post-test 2		
Knowledge	MS	±SD	t.	MS	±SD	t.
Study group	33.17	4.227	9.462	32.70	4.622	8.977
Control group	23.60	3.578		23.67	3.614	
	P-value =.007			P-value =.015		

Table 9. A comparison between mothers practices with the study and control groups at post-tests (1 and 2).

Groups	Post-tes1			Post-test 2		
Practices	MS	±SD	t.	MS	±SD	t.
Study group	40.03	6.261	9.067	38.13	6.474	7.395
Control group	27.83	3.887		27.90	3.942	
	p-value .006			p-value .018		

DISCUSSION

The findings of the present study show that most of the caregivers in the control and study groups were patients' mothers. This result was supported by a study which found that (65.7%) of Indonesian acute leukemic caregivers were patients' mothers [13] . and with other was done by Al- Jaussy, in Jordan 2010 who found that the caregivers represented (76% and 78%) in the control and study groups respectively were mothers' patient [(15)]. Another study done by Saeui *et al*, in 2009 and supported by others which found that about (77%) of caregivers were females and patients' mothers [14,16].

The mean age of caregivers was (41.7±9.176 and 40 ± 8.15) years old in the control and study groups respectively. This result was in agreement with a study done by Aziz who conducted a health educational program on children's mothers with acute leukemia in Baghdad city and found that most of the mothers' age were (40 years or older) for both the control and study group [16] . Another study found that the caregiver's age was more than (41) years old and represented (54% and 66%) in the control and study groups respectively [17].

The results of present study found that one third of caregivers were illiterate. This result was supported by a quasi-experimental study was done in 2007 and found that the highest percentage (40%) of participants were illiterate in the both control and study groups [18]. In contrast to the result others found that (66.7%) of participants had a minimal education level of primary school [16,19] . but the present result is in disagreement with studies who found that most of the caregivers were graduates from secondary schools or above [13,15,18] .

Most of the caregivers were from rural areas. This result is in agreement with a study who found that (79.3%) of acute leukemic children were coming to the hospital from outside the city [20]. The present study found that the majority of caregivers in the control and study groups respectively were living with low SES. This result is in agreement with studies which found that two third of acute leukemic patients in the control and study groups respectively were from low SES [5,13,15,21].

The mean ages of adolescent leukemic patients in the study and control groups were (14.94 ± 2.950, and 14.83±3.535) years old respectively. This result is in agreement with a study which conducted on 132 adolescent patients with variance types of cancer and found that the age group was (15.75 SD=1.78) years old, and also supported by other studies which found that the mean age of adolescent leukemic patients (12.9± 1.6 and 13.57 ±1.75) years old [5; 22]. Others found that their ages were between 2 to 14 years old [13].

Most of the adolescent leukemic patients in the study and control group were males. Others found that the (65.8%) of adolescents were males [13] . the present study found that most of the adolescent leukemic patients in the control and study groups were having type ALL respectively. Others found that the acute leukemia represented (72.6%) among adolescents' age [23].

Throughout the use of (Independent t-test and ANOVA) the result indicated that there were no statistically significant differences between control and study groups related to pre-test, and there were no significant differences between pre and post-tests (1 and 2) regarding knowledge and practices of caregivers in the control group. This result indicates that the caregivers of leukemic adolescent had lack knowledge and poor practices toward patient care before the implementation of the health educational program. This result is in agreement with a study done in Hong Kong and with another study was done in Shiraz (Iran), found that there was no significant difference between the control and study groups before the implementation of the health education program and there were no significant differences between pre and post-tests in the control group [17:23].

After the implementation of the health education program, the present study found that there was a significant difference between pre and post-tests (1 and 2) in the study group regarding to the caregivers knowledge and practices. The result shows that the caregivers improved their knowledge and practices in the post-tests (1 and 2). This result was supported by a quasi-experimental intervention by Given *et al*, in 2003 and found that the caregivers improved their knowledge and practices towards cancer patient management [24] **.** Another study was done in Iran –Shiraz their aim was to improve the knowledge and practices of caregiver having oncology patients, and finding of the result found that there was a significant difference between pre and post-tests in the study group at p-value (.001) after 2 months of the health educational intervention [23] **.**

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CONCLUSIONS

The study concluded that the majority of caregivers were mothers patient, above (41) years of age, illiterate, coming from rural areas, living in low SES, and the study found that there is no significant differences between control and study group before health educational program intervention, but found that there is significant differences between pre and post tests (1 and 2) regarding knowledge and practices of caregivers having leukemic patients, and feature efforts should focus on longitudinal studies that

describe their physical problems over time within individual patients.

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پوختە

کاریگەری برنامەی فیزکاری تەندروستی لەسەر زانیاری و کرداری بەخێوکارانی گەنجای تووش بوو بە لۆکیمیای تیژ

پێشەکی و ئارمانج: لۆکیمیا بریتی یە لە شێر پەنجەی خوێن و بەوە دەناسرێتەوە کە مۆخی ئێسك یان بەشەکانی تری لەش بە شێوەیەکی ناکام خروکە سپیەکان دروست دەکەن، ئەمرۆ خزمەت کردن لە ماڵەوە بەخێرایی گەشە دەکات شان بەشانی خزمەتە تەندروستیەکان . بەخیوکاران روڵی گرنگیان هەیە لە ئاگاداری دەرونی و جەستەیی توشبوان بە شێرپەنجە.

ئارمانج: ئەم لێکولینەوەیە بە ئامانجە کە زانیاری و کرداری بەخێوکاران چالاك و زیاد بکات کە نەخۆشی توش بوو بە لۆکیمیایان هەیە.

ڕێکێن ڤەکولینێ: لێکۆلینەوەیەکی نیمچە ئازمایشتی یە لە نەخۆشخانەی نانەکەلی بۆ نەخۆشیەکانی خوێن لە بەرواری 1 \ تشرینی یەکەمی \ 2010 تا 1 \ تشرینی یەکەمی \ 2011 ئەنجام دراوە. حەفتا بەخێوکار بانگران بۆ لێکولینەوەکە وە دابەش کران بۆ دوو گروپی یەکسان (35 گروپی توێژینەوە) (35 گروپی کۆنترۆل) .

ئەنجام: زۆربەی بەخێوکاران دایکانی نەخۆشەکە بوون و ناوەندی تەمەنیان (41.7±9.176 و 40 ± 8.15) ساڵ بوو زۆربەیان نەخوێندەوار بوون و لە دەرەوەی شاری هەولێر هاتبوون، لە بارێکی کۆمەلایەتی –ئابوری نزم دا دەژیان. ناوەندی تەمەنی گەنجە تووش بوەکان (14.83±3.535و 14.94 ± 2.950), ساڵ بوون و زۆربەیان کوڕ بوون و هەڵگری جۆری ALL بوون، لە هەردوو گروپی توێژینەوەو کۆنترۆڵدا . ئەنجامەکان دەریانخست کە جیاوازیەکی ئاماریی بەرز هەیە سەبارەت بە زانیاری و کرداری بەخێوکاران لە هەردوو تاقیکردنەوەی پێشی و پاشی (1 و 2) دا بەشێوەی (.001, 028, 003 .012) .

دەرئەنجام: دەرئەنجامەکان ئاماژە بە زیادبوونی زانیاری و کرداری بەخێوکاران دەکات لە گروپی توێژینەوەدا لە پاش پێشکەش کردنی پرۆگرامی تەندروستی فیزکاری .

الخلاصة

تأثير البرنامج التثقيفي الصحي على ممارسات و معلومات المقدمى الرعاية الذين لديهم مرضى المصابين باللوكيما الحادة

خلفية البحث: اللوكيميا هو سرطان الدم الذي يتميز بنموا خلايا دم بيضاء غير ناضجة و الذى ينتجها النخاع العظم او اعضاء اخرى من الجسم . اليوم تنمو و بسرعة الرعاية المنزلية تزامنا مع الرعاية الصحية ، ان مربي الرعاية لها الدور كبير في العناية النفسية و الجسدية للمرضى السرطان.

الاهداف: يهدف البحث الى تطوير الممارسات و المعلومات لمربي الرعاية الذين يقدمون العناية المنزلية للمراهقين المصابين باللوكيميا.

طرق البحث: أجريت دراسة شبه تجربية في مستشفى نانه كه لي لامراض الدم في محافظة اربيل للفترة ما بين 1 \ تشرين الاول \ 2010 الى 1 \ تشرين الاول 2011 \ ، اختيرت عينة (70) مربيا و قسمت الى مجموعتين متساويتين (35) مجموعة الضابطة و (35) مجموعة أختبارية.

النتائج: أظهرت النتائج بان معظم مربي الرعاية كانوا امهات المرضى، معدل اعماره (14.94 ± 2.950 و 14.83±3.535) سنة، نسبة عالية كانوا من الأميين، جاءوا من خاج مدينة اربيل، يعيشون فى وضع اقتصادي – أجتماعي واطيء. معدل اعمارمرضاهم المصابين (14.94 ± 2.950 و 14.83±3.535) سنة ، اكثيرهم كانوا ذكورا ، و مصابين بمرض البعدي للمجموعة السرطان بنوع ALL ، في كلا المجموعتين الضابطة و الاختبارية بالتتابع. كما اظهرة النتائج بان هناك فرق ذو دلالة احصائية عالية بين الاختبار القبلي والبعدي (1 و 2) للمجموعة الاختبارية المتعلقة بممارسات و معلومات مربي الرعاية (.001 0 .028 0 .003 0 و .012 0).

الاستنتاجات: اظهرت النتائج بتحسن في ممارسات و معلومات لملربي الرعاية للمرضى المراهقين و المصابين بالوكيميا الحادة بعد تطبيق البرنامج التثقيفي الصحي للمجموعة الاختبارية.

ERYTHROCYTE DELTA-AMINOLEVULINC ACID DEHYDRATASE LEVELS AMONG THE GENERAL POPULATION OF DUHOK GOVERNORATE, KURDISTAN REGION, IRAQ.

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ABSTRACT

Background and objectives: Lead is a potent inhibitor of delta –aminolevulinc acid dehydratase (ALAD), enzyme that catalyzes the second step in the biosynthesis of heme. Because the metal has the greatest effect on ALAD, measurement of ALAD activity can be used as a marker of lead exposure. Therefore, in this study, we investigate the degree of lead exposure by ALAD activity in the general population of the Duhok Governorate.
Methods: A cross-sectional study was conducted on 1340 individuals, not occupationally exposed to lead (n=820) and occupationally exposed to lead (n= 520). In all the individuals we measured blood concentrations of lead and ALAD levels.
Results: The mean (+SD) ALAD was 154+31.1 unit/ml erythrocytes. Of the 1340 individuals tested, 253% (n=340) had ALAD values of <130 unit/ml erythrocytes (lower-first quartile). The mean ALAD values were significantly ($p<0.001$) lower for the occupational group as compared to the non-occupational group. Blood lead levels showed a negative linear relationship with ALAD levels($r= -0.59$, $p<0.001$).
Conclusions : ALAD measurements reflects an increased lead exposure among occupationally exposed individuals which was accurately and closely related to lead concentrations in blood

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Key words: Lead exposure, erythrocytes ALAD

Lead is inhibitor to the enzymes that catalyze the biosynthesis of haem. The metal is known to induce anemia in subjects with heavy exposure [1] Environmental exposure to lead is associated with arterial hypertension, kidney involvement, endocrine, immune system and hematological disorders [2,3,4,5] .The only efficacious method for avoiding these toxic effects is to control the population's exposure to lead by irradiating its sources which may not always be obvious. Hence it is essential to know the level of exposure of the population so that necessary control measures can be established. The best test for determining lead-exposure status is blood test, which is an indirect of exposure to the metal [6]. ALAD determination had previously recommended as a screening technique for environmental exposure to lead., since lead exposure provokes decreased ALAD values due to effects of the metals on haem biosynthesis [7] . This method has the advantage of being easy to perform and low level of exposure would be detected.. The aim of this was to determine lead exposure in a sample of the general population from Duhok Governorate by using ALAD test.

METHODS

This study was conducted between 1 January and 31 Decmber2010 in the Department of clinical Biochemistry, School of Medicine, University of Duhok. In order to provide a wide range of lead exposure, one sample with substantial environmental exposure (Occupationally

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exposed to lead, they were workers of Gasoline generator, General Industrial, Traffic Policemen, Petrol storage and Battery repairing) from two industrial areas, namely Baroshky and Malta were selected and one sample with lower exposure (general population who were not occupationally exposed to lead) were selected on the basis of residence and occupation from Duhok city center and Duhok city quarters. A total of 1340 individuals who were at 1-64 years old, and who represented a random sample of the population were recruited. Data on age, gender, residence, current occupation and its duration in years, cigarette smoking and symptoms frequently encountered in lead exposure. A total of 1340 blood samples were analyzed. Blood analysis included blood lead level and erythrocytes ALAD activity was done according to the previously published methods [8, 9].All data were analyzed using the Statistical Package for Social Science SPSS version 18; paired student t-test was used to assess differences in blood analyte among groups. Significance of distribution of ALAD was assessed using Chi-square test. Level of statistical significance (p-value) was set at <0.05. The results were plotted on Receiver Operator Characteristic (ROC) curve, which graphically represent the relationship between sensitivity and specificity for different cut-off points [10].

RESULTS

Mean ALAD value in the sample was 154.0 unit/ml erythrocytes, with a standard deviation of 31.1 and a minimum value 96 unit/ml RBC. The distribution histogram of blood values in the sample is illustrated in (Figure 1).A significant difference was observed for the occupational group(146.5 unit/ml erythrocytes) compared with non-occupational group(162.4 unit/ml erythrocyte), results are shown in (Table 1).The relationship between blood lead and ALAD is shown in (Figure2) . Blood lead levels showed a negative linear relationship with ALAD levels(r= -0.59, p<0.001). The distribution of ALAD levels and blood lead concentrations are shown in (Table 2). Of the 1340 individuals tested, 25.3% (n=340) had ALAD values of <130 unit/ml erythrocyte (lower-first quartile), which showed 19.7 %(n=264) had blood lead concentration > 10 ug/dl. The usefulness of ALAD determination as a screening test for lead exposure was assessed by calculating its sensitivity and specificity for different ALAD cut-off points. Maximum values were established at the cut-off of 144.5 unit/ml erythrocytes which showed 77.3% sensitivity and 79.2% specificity. The extrapolation of data to a ROC curve is shown in (Figure 3).

Table 1. ALAD and blood lead levels in the sample

Group	n	ALAD (unit/ml erythrocytes) Mean+SD	Range	Blood lead (ug/dl) Mean+SD	Range
Occupational	**520**	***146.5+11.6**	**96-176**	***14.5+6.2**	**3.2-55.3**
Non-occupational	**820**	**162.4+18.3**	**100-264**	**7.3+2.8**	**1.6-17.0**
Total	**1340**	**154.0+31.1**	**96-264**	**10.1+7.4**	**1.6-55.3**

Occupational Vs non-occupational, P<0.001

Table 2 .Distribution of ALAD values according to individuals' blood lead

ALAD(unit/ml erythrocytes)	n	Blood lead concentration(ug/dl)			P-value
		<10	>10-25	>25	
		n (%)	n (%)	n (%)	
Lowest (First quartile)(<130)	340	76 (22.4)	213(62.6)	51(15.0)	<0.001
Average (Inter-quartile) 131-170	675	398(59.0)	277(41.0)	0(0.0)	
Highest (Fourth quartile)(>171)	325	325(100.0)	0(0.0)	0(0.0)	
Total	1340	799(59.6)	490(36.6)	51(3.8)	

All individuals with blood lead levels over 10ug/dl were considered exposed.

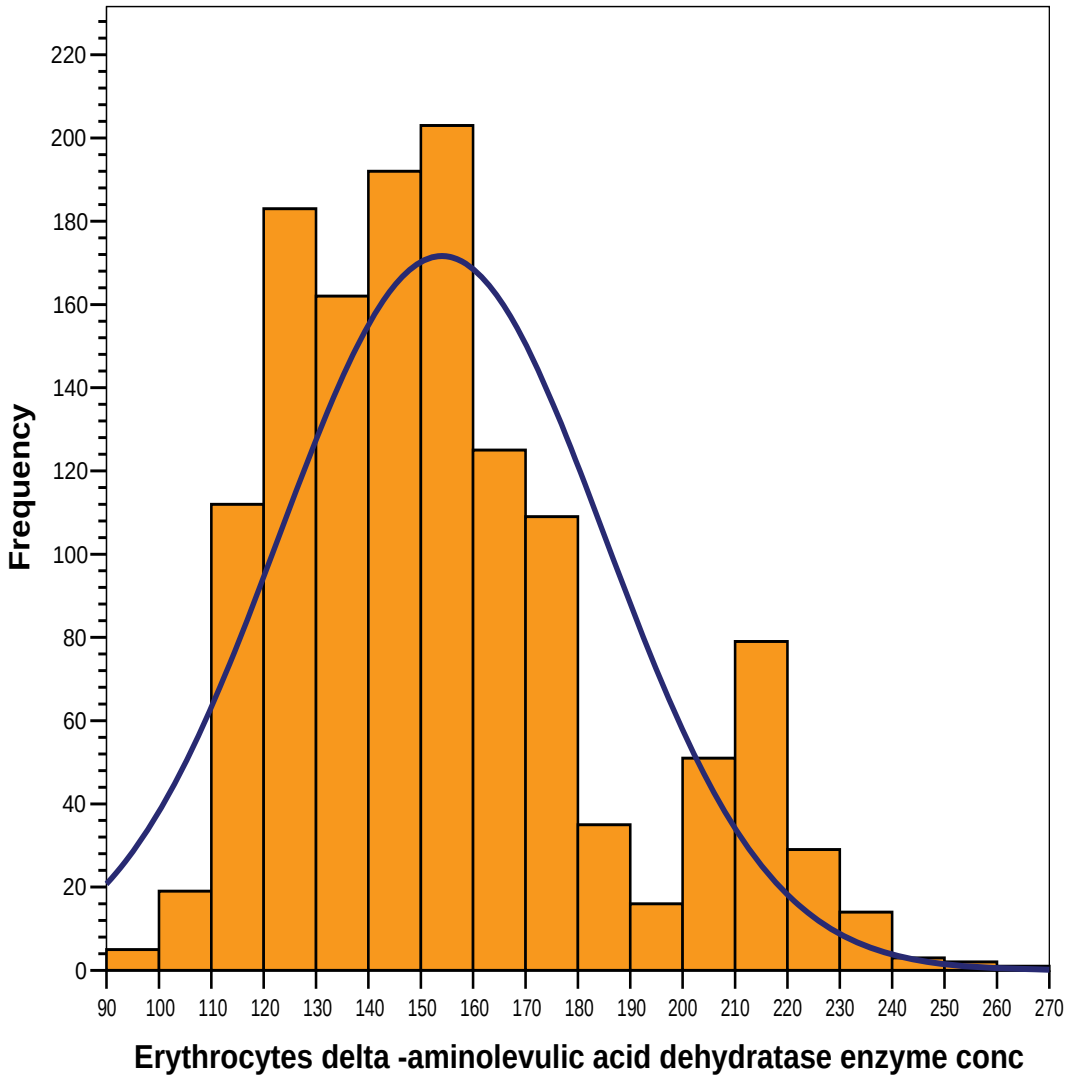

Figure 1. Distribution histogram of ALAD values in the sample

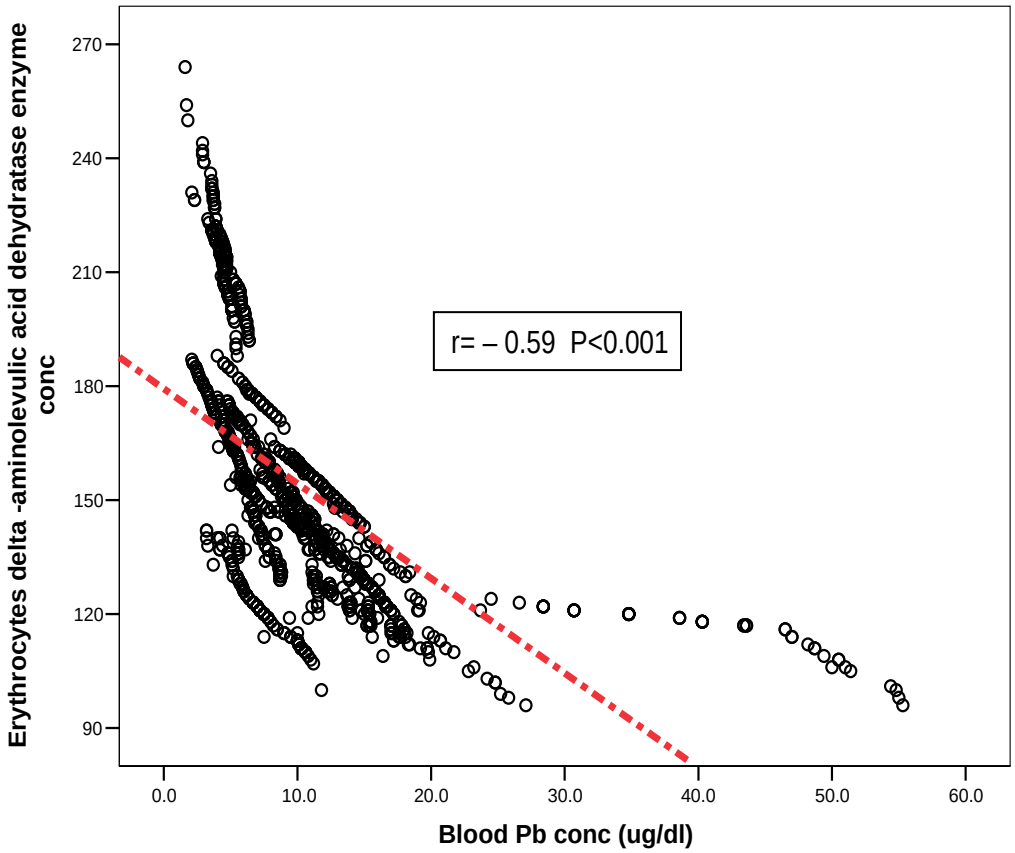

Figure 2. Scatter diagram with fitted regression line showing the linear correlation between ALAD and blood lead in the sample

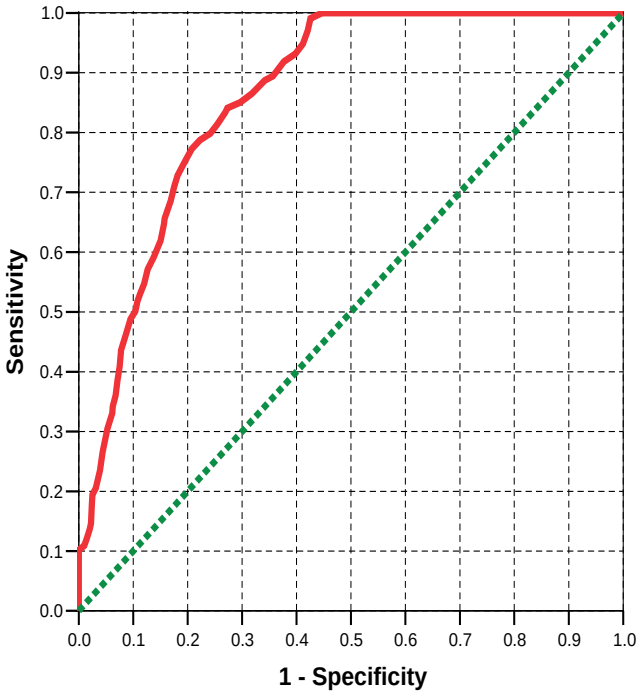

Figure 3 .Correlation between sensitivity and specificity for different ALAD cut-off points. The area below the curve significantly differs from the null value curve (dotted line) Area below the curve=0.832, IC 95%:0.762-0.882.

DISCUSSION

The use of erythrocytes ALAD had previously been recommended as an exposure marker, since lead exposure provokes decreased ALAD values due to effects of the metal on enzyme activity [11] . This method has the advantage of being easy to perform and detect lead exposure at low levels. But in contrast these decreases in ALAD being from blood lead values above 10ug/dl, and a level below 10ug/dl would not detect. In fact, 77.6% of the individuals in this study presented ALAD values within the lowest-first quartile (ALAD<130 unit/ml erythrocytes) which associated with blood lead levels over than 10 ug/dl and 15% associated with blood lead levels over than 25 ug/dl. There was a tendency towards decreased ALAD values with the increase in blood lead levels; inverse statistically-significant linear relationship was observed. ALAD cut-off point 144.5 unit/ml erythrocytes presented acceptable sensitivity and specificity for detecting individuals with lead exposure at a levels more than 10 ug/dl. The area below the ROC curve supports these finding. Thus ALAD determination appears to be a useful method for detected individuals within the permissible blood lead levels reported by others [12]. Other authors have also demonstrated the usefulness of ALAD determination as a screening test for lead exposure in general population [13, 14] . The values of ALAD are correspondent to the blood lead levels obtained in both occupationally exposed and non-occupationally exposed population. Anderson et al, 1996 [15] considered 50% reduction in ALAD activity is an indication of lead toxicity. In the present study, none of the non-occupationally exposed individuals showed such a low percentage of reduction, however, 25.3% of the overall population had low ALAD values, and most of them were occupationally exposed workers In conclusion, body burden of lead measured by ALAD levels reflects an increased lead exposure among occupationally exposed individuals which was accurately and closely related to lead concentrations in blood

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پوختە

خەملاندنا کارتێکرنا ئەنزیمێ ئەمینو لیڤولینك اسید دیهایدریت ALAD ل دەف خەلکێ پارێزگەها دهوکێ – هەرێما کوردستانێ.

پێشەکی وئارمانج: توخمێ رصاصی کەرەستەکێ سەرەکی یە بو کێمکرنا کارتێکرنا ئەنزیمێ ALAD ل پێنگاڤا دووێ یا پیّك هینانا هیموگلوبینی چونکە کانزایی ئاسنێ کارتێکرنەکا زور هەیە ل سەر ئەنزیمێ ALAD.

ریّکێن ڤەکولینێ: ئەڤ ڤەکولینە لسەر 1340 بەشداربویا هاتی یە کرن ول سەر دوو گروپێن سەرەکی دابەش بوینە ، (820) بەشدار بەرهنگاری توخمێ رصاصی بوینە و (520) بەشدار بەرهنگاری توخمێ رصاصی نەبوینە. خەملاندنا توخمێ رصاصی وکارتێکرنا ئەنزیمێ ALAD بو هەمی بەشداربویا هاتی یە کرن دناڤ خوینێ دا.

ئەنجام: دئەنجامدا دیاربو کو تێکرایا کارتێکرنا ئەنزیمێ ALAD 154 unit/ml بو هەمی بەشداربویا تێکریا کارتێکرنا ئەنزیمێ ALAD لدەف بەشداربویێ بەرهنگاری توخمێ رصاصی بوین کێمتر بو بەشدارێن بەرهنگاری توخمێ رصاصی نەبوین وجاوازی یا دیاربو د پێڤەرێن ئاماریّ دا کود بیتە $P<0,01$

دەر ئەنجام: نزمبونا تێکرایا کارلێکرنا ئەنزیمێ ALAD دیاربت بو زێدەبونا ریژەیا توخمێ رصاصی بو بەشدارێن بەرهنگاری توخمێ رصاصی بوین لەورا دڤێت ریکێن ساخلەمیێ بێنە وەرگرتن دا کو ریژا توخمێ رصاصی دناڤ خوینێ دا زێدە نەبیت.

الخلاصة

تقدير فعالية انزيم امينو ليفولينك اسيد ديهايدريت (ALAD) عند عامة الناس في محافظة دهوك – اقليم كوردستان

خلفية الموضوع: الرصاص مادة اساسية تعمل على تنشيط فعالية انزيم ALAD في الخطوة الثانية من تفاعلات تخليق الهيموكلوبين وذلك لأن فلز الحديد له تأثير كبير على فعالية انزيم (ALAD).

طرق البحث: شملت الدراسة (1340) مشاركاً مقسمين الى مجموعتين هما (820) مشاركاً من غير المعرضين مهنياً للرصاص و (520) مشاركاً من المعرضين مهنياً للرصاص. وتم تقدير تركيز الرصاص وفعالية انزيم ALAD لجميع المشاركين في عينات الدم.

النتائج: أظهرت النتائج بأن معدل فعالية انزيم ALAD كان 154 unit/ml لكل المشاركين وكان معدل فعالية الأنزيم عند الاشخاص المعرضين مهنياً للرصاص أقل من الأشخاص الغير المعرضين مهنياً للرصاص والفرق كان ذو قيمة احصائية وبلغت $P < 0,01$.

الاستنتاجات: انخفاض معدل فعالية انزيم ALAD يدل على زيادة نسبة تركيز الرصاص عند الاشخاص المعرضين مهنياً للرصاص. لذا يجب اتخاذ تدابير الرقابة والسلامة العامة للحد من ارتفاع مستوى تركيز الرصاص في الدم.

PREVALENCE OF THYROID DYSFUNCTION IN PATIENTS WITH METABOLIC SYNDROME

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ABSTRACT

Background and Objective: The metabolic syndrome (MS) and thyroid dysfunction are independent risk factors of atherosclerotic cardiovascular disease; coexistence of the two will substantially increase cardiovascular risk. We aim in this paper to report on patients with the metabolic syndrome a) the prevalence of thyroid dysfunction b) the association between thyroid hormones and metabolic syndrome components.

Methods: One-hundred patients who attended Duhok Diabetes Center during the period of the study and confirmed to have the metabolic syndrome were enrolled in this study. The study was conducted between 1st November 2010 and 30th June 2011. The diagnosis of the metabolic syndrome was based on the criteria set by the American National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III). The anthropometric measurements were done and fasting blood samples were analyzed for free triiodothyronine (free T3), free thyroxine (free T4), thyroid stimulating hormone (TSH), lipid profile, insulin, and glucose and these values were compared with those in a control population (n = 100).

Results: The prevalence of thyroid dysfunction was significantly higher among MS patients as compared to control subjects. Of the one-hundred patients with MS, 77% were euthyroid, 19% had subclinical hypothyroidism and 4% had overt hypothyroidism. With respect to the control subjects, 96% were euthyroid and 4% had subclinical hypothyroidism. Hyperthyroidism was not present in any of the participants. Free T3 and free T4 were significantly lower in euthyroid MS patients than in the controls ($P < 0.01$). Thyroid stimulating hormone (TSH) correlated positively with the number of metabolic syndrome components ($P < 0.05$). Free T3 and free T4 did not correlate with metabolic syndrome components.

Conclusions: It can be concluded that metabolic syndrome patients have a higher prevalence of thyroid dysfunction which might predispose them to cardiovascular events.

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Key words: thyroid dysfunction, metabolic syndrome.

The metabolic syndrome is a common health problem. It is a widely prevalent and multi-factorial condition. This syndrome constitutes a cluster of risk factors characterized by hypertension, atherogenic dyslipidemia, hyperglycemia, and prothrombotic and proinflammatory conditions [1].

Obesity, insulin resistance, physical inactivity, advanced age and hormonal imbalance have been suggested as the underlying risk factors for the development of this syndrome [2]. Of these hormones, thyroid hormones which play an essential role in regulating energy balance and metabolism of glucose and lipids may have effects on the MS [3]. Concerning the relationship between MS and thyroid dysfunction, several studies reported that lower free T4 levels are associated with unfavorable lipid levels and higher insulin resistance [4-6].

However, reports on the relationship between this syndrome and thyroid

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dysfunction are inconsistent and limited, especially locally.

Because of this lack of information regarding thyroid status and its relation with MS components among our local population, we were prompted to conduct the current study to determine the prevalence of thyroid dysfunction in a sample of patients with the metabolic syndrome.

METHODS

This case-control study included 100 patients (both men and women) aged between 35 and 60 years with confirmed metabolic syndrome and 100 apparently healthy participants with no history of thyroid diseases were enrolled to serve as controls. The patients were from Duhok Diabetes Center and the controls were from staff and substaff of Azadi General Teaching Hospital, Duhok, Kurdistan Region, Iraq.

The study protocol was approved by the ethics committee of Directorate of Health-Duhok and informed consent was obtained from all the participants at the start of the study. The diagnosis of metabolic syndrome was based on the criteria set by the American National Cholesterol Education Program-Third Adult Treatment Panel (NCEP-ATP III) [7] .

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 triglyceride 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 or on pharmacological treatment for hypertension and fasting blood glucose level of 100mg/dl or greater.

Patients with a history of kidney disease, liver disease, pregnancy, recent surgery or taking medication that can alter thyroid hormone levels were excluded. At the baseline, the anthropometric data including waist circumference and blood pressure were measured and a detailed physical examination was done. The blood pressure was measured in the right arm in the supine position; three readings were taken and the mean value of the three readings was taken as the final recording. The waist circumference was measured by a tape measure at the plane between the anterior superior iliac spines and the lower costal margins at the narrowest part of the waistline while the participant was standing and during slight expiration.

The participants were asked to fast overnight for at least 12 hours. At the morning, venous blood samples were collected and serum glucose, triglycerides, HDL-cholesterol and insulin level were determined.

Serum TSH, free T3 and free T4 were also measured. Insulin resistance was estimated by using homeostasis model assessment for insulin resistance (HOMA-IR) by the equation:

HOMA-IR=[(FBG mgdlx0.05)x(insulin level µIU/ml)]/22.5.

A participant was considered to have insulin resistance if HOMA-IR was >1.69.

A high serum TSH (> 4.5 - 10 µIU/ml) and a normal or slightly lowered free throxine (FT4) level were required for the diagnosis of sub-clinical hypothyroidism. The patients were classified as overt hypothyroidism when the TSH level was >10µIU/ml and when the FT4 level was low (FT4 < 0.93 ng/dl). Patients with normal TSH (0.25-4.5 µIU/ml) and FT4 (0.93-1.7) ng/dl levels were considered to be euthyroid.

The data are presented as Mean+SD for continuous variables. The Student's t-test was applied for comparison of group means. Pearson's coefficient of correlation was calculated to determine the correlation between 2 variables. P value of less than 0.05 was considered statistically significant.

RESULTS

The general characteristics of the study sample are described in (Table 1). Of the hundred patients, 4% had overt hypothyroidism, 19% had sub-clinical hypothyroidism and 77% were euthyroid. With respect to the controls, 96% were euthyroid and 4% had sub-clinical hypothyroidism, whereas none of the participants was hyperthyroid. The mean TSH level of the MS patients was 2.6±1.7µIU/ml and of the healthy controls was 1.8±1.1µIU/ml, the difference was statistically significant (P < 0.01). Patients with MS had lower serum free T3 and free T4 levels than healthy controls (P < 0.05, for all parameters).

Table 1. Baseline characteristics of patients and controls

Characteristic	Patients (n=100)	controls (n=100)	p-value
Age (years)	50.0±7.4	48.0±6.5	NS
Male gender n (%)	34(34%)	36(36%)	NS
Waist circumference			
Males	104.9±6.6	87.1±10.6	<0.01
Females	102.2±9.5	81.6±4.8	<0.01
Systolic BP (mmHg)	133.1±21.1	114.9±7.3	<0.01
Diastolic BP (mmHg)	84.8±9.4	75.0±6.4	<0.01
Fasting blood glucose(mg/dl)	200.3±76.0	86.8±4.8	<0.001
Triglycerides (mg/dl)	214.5±85.5	94.3±36.2	<0.001
HDL-Ch (mg/dl)			
Males	36.1+4.3	40.4+4.3	<0.05
Females	39.0+6.9	46.3+5.3	<0.05
Fasting serum insulin (uIU/ml)	8.9+ 2.6	7.0+1.2	<0.01
HOMA-IR	4.0+2.1	1.3+0.2	<0.001
TSH (uIU/ml)	2.6+1.7	1.8+1.1	<0.01
Free T3 (pmol/l)	13.0+2.8	14.9+2.4	<0.05
Free T4 (pmol/l)	5.0+ 1.0	6.0 +1.1	<0.05

Data are expressed as X+SD, percentage or as indicated

(Table 2) shows the mean values of thyroid related hormones in the euthyroid patients and the euthyroid controls. The mean values of free T4 and free T3 significantly differ between the two groups, whereas no significant difference was observed in TSH values. There were significant differences between euthyroid patients and euthyroid controls in waist circumference, blood pressure, level of fasting blood glucose, indices of insulin resistance (fasting insulin level, HOMA-IR) and lipid profile.

All MS patients were grouped into three sub- groups according to the number of the MS components as 3 components, 4 components, and 5 components (Table 3). The mean TSH-specific levels in the three groups were 2.1±0.4, 2.5±0.3 and 3.3±0.5 respectively. Patients with 5 components had higher TSH levels as compared to the other component groups, but the difference was statistically nonsignificant (P = 0.23).

(Table 4) shows the correlation between thyroid related hormones and metabolic syndrome components. Although the level of TSH was not correlated with waist circumference , blood pressure, serum triglycerides , HDL-cholesterol, glucose, fasting and HOMA-IR; the number of metabolic syndrome components was significantly correlated with TSH level (r = -0.27, P = 0.045). Free T3 and T4 levels did not correlate with any component of metabolic syndrome.

Table 2. Thyroid related hormones and metabolic characteristics of euthyroid patients and euthyroid controls.

Variable	patients (n=77)	controls (n=96)	p-value
Waist circumference (cm)			
Males	**104.3±6.8**	**87.3±10.8**	**<0.01**
Females	**101.6±9.8**	**81.6+ 4.6**	**<0.01**
Systolic BP (mmHg)	**134.2±19.7**	**114.9+7.3**	**<0.01**
Diastolic BP (mmHg)	**84.9±9.1**	**75.0+6.2**	**<0.01**
Fasting blood glucose(mg/dl)	**199.0±75.2**	**86.8±4.8**	**<0.001**
Triglycerides (mg/dl)	**211.3±84.0**	**94.5±36.6**	**<0.001**
HDL-Ch (mg/dl)			
Males	**37.1+4.3**	**40.5±4.4**	**<0.05**
Females	**39.6±7.2**	**46.1±5.1**	**<0.05**
Fasting serum insulin (uIU/ml)	**8.7± 2.7**	**6.9±1.1**	**<0.01**
HOMA-IR	**3.9±1.9**	**1.3+0.2**	**<0.001**
TSH (uIU/ml)	**1.7±0.8**	**1.6±0.8**	**0.073**
Free T3 (pmol/l)	**13.6±2.8**	**15.1±2.2**	**<0.05**
Free T4 (pmol/l)	**5.2±1.0**	**6.1±1.0**	**<0.05**

Data are expressed as X±SD.

Table 3. Thyroid related hormones in MS patients categorized by the number of MS components

Variables	3 components (n=30)	4 components (n=39)	5 components (n=31)	p-value*
TSH	**2.1+0.4**	**2.5+0.3**	**3.3+0.5**	**0.23**
FT3	**5.2+0.1**	**4.9+0.1**	**4.9+0.1**	**0.38**
FT4	**13.4+0.3**	**13.1+0.3**	**12.5+0.3**	**0.28**

Data are presented as X+SD
**Based on One-way ANOVA test.*

Table 4. Correlation between thyroid related hormones and MS components

	TSH		FT3		FT4	
MS components	**r**	**p-value***	**r**	**p-value***	**r**	**p-value***
Waist circumference (cm)						
Males	**0.217**	**0.218**	**-0.300**	**0.085**	**-0.092**	**0.604**
Females	**0.104**	**0.406**	**-0.147**	**0.238**	**-0.027**	**0.828**
Systolic BP (mmH g)	**0.052**	**0.609**	**0.120**	**0.235**	**0.130**	**0.197**
Diastolic BP (mmHg)	**0.005**	**0.960**	**0.076**	**0.452**	**0.043**	**0.670**
Fasting blood glucose(mg/dl)	**0.036**	**0.720**	**-0.077**	**0.446**	**0.149**	**0.140**
Triglycerides (mg/dl)	**0.090**	**0.367**	**-0.125**	**0.215**	**-0.107**	**0.290**
HDL-Ch (mg/dl)						
Males	**-0.054**	**0.760**	**-0.202**	**0.252**	**-0.012**	**0.946**
Females	**-0.241**	**0.052**	**0.246**	**0.051**	**0.163**	**0.191**
Serum insulin (uIU/ml)	**0.085**	**0.403**	**0.027**	**0.793**	**-0.152**	**0.130**
HOMA-IR	**0.063**	**0.530**	**-0.065**	**0.522**	**0.043**	**0.671**
Number of components	**0.270**	**0.045**	**-0.109**	**0.279**	**-.0152**	**0.132**

r: Pearson correlation coefficient (2-tailed)
** Based on t-test (2-tailed)*

DISCUSSION

Several studies have shown a fascinating link between the thyroid related hormones and some specific components of the metabolic syndrome in euthyroid women [8]. Both thyroid dysfunction and metabolic syndrome are independent risk factors of atherosclerotic cardiovascular disease [9]. This has potential health-care implications, because of possible widespread low thyroid function and increased risk of metabolic syndrome in our local area. However, reports on this issue, at least in our population, are limited [10]. The present study indicated that thyroid hypofunction was more among the metabolic syndrome group. Low thyroid function was present in 23% of the cases. In the control group 4% had sub-clinical hypothyroidism and none of them had overt hypothyroidism. In a previous study by Jayakumar et al, it was reported that 60 percent of the cases with metabolic syndrome had thyroid function abnormalities in their case series [11]. A more recent study revealed that the prevalence of thyroid dysfunction was more among the females with metabolic syndrome. Sub-clinical hypothyroidism was present in 53% of the cases and overt hypothyroidism was present in 25% of the patients [12]. In comparing the present findings with those of other studies, our results exhibit controversial outcome to previous studies. Nevertheless, the present study has shown a strong association between sub-clinical hypothyroidism and metabolic syndrome. This trend indicates a significant link between thyroid dysfunction and metabolic syndrome. Similar to our observation, the study by Uzunlulu et al, had shown sub-clinical hypothyroidism prevalence to be 16.4% (n=36) in the metabolic syndrome group (n=220) (3). However, the other authors did not address men in their studies and all observations were on women only. The present study found that 15 out of 66 females with metabolic syndrome (22.6%) had sub-clinical hypothyroidism. With respect to male's thyroid status, 4 out of 34 metabolic syndrome males (11.7%) had sub-clinical hypothyroidism. A previous review has identified the prevalence of sub-clinical hypothyroidism to be 4% to 8% in the general population, and up to 15% in women who were over 60 years of age in the western population [13]. However, data is lacking with respect to the prevalence of hypothyroidism in our population. Our results were comparable with those of the above mentioned study; 4% of the controls had subclinical hypothyroidism.

A previous meta-analysis indicated that the prevalence of thyroid disease in patients with diabetes is significantly higher than that in the general population [14]. The present case-control study reports on the thyroid status of male and female patients with metabolic syndrome affected by type 2 diabetes mellitus. Our results were comparable with those of the above mentioned study and revealed a varied effect of thyroid status on the components of metabolic syndrome: waist circumference, blood pressure, triglycerides, HDL-cholesterol, glucose and HOMA-IR. The observed change was statistically significant between the euthyroid patients and euthyroid controls. This can be explained through the metabolic pathway. within the metabolic syndrome cluster, there are several mechanisms through which one abnormality could favor for the development of the other [15]. In the present study, mean systolic pressure, diastolic pressure, waist circumference, fasting blood sugar, triglycerides and TSH values were significantly higher in the metabolic syndrome group compared to the control group. This study has provided evidence for the first time that our population belonged mainly to metabolic abnormalities was with high prevalence of thyroid dysfunction. Further cross-sectional and longitudinal studies with larger number of patients are required to confirm these findings.

The limitation of this study was that the study population consisted of only the patients who visited Duhok Diabetes Center during the period of the study. Therefore, the data of this study do not reflect that in the general population. Another point in this study is limited by a small sample size, and the results must be confirmed in a large sample. Another point of consideration is that insulin resistance has been measured indirectly only and we did not examine insulin resistance of the subjects using standard methods like hyperinsulinemic euglycemic clamp even in sub group analysis. Even though the present study indicated that the thyroid hypofunction shows the association with metabolic syndrome components.

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پوختە

ڕێژا بەربەلاڤبونا تێکچونا کارێ ڕژینا ستوی (ڕژینا ئەندەرەقییە) ل دەف کەسێن توشی نەخوشیا دیاروکا ژینکار بووین

پێشەکی: دیاروکا ژینکار و تێکچونا کارێ (Dysfunction) ڕژینا ستوی (ڕژینا ئەندەرەقییە) دوو فاکتەرێن سەربەخونە یێن مەترسیێ ب توشبونا نەخوشیێن دلی ودەمارێن خوینێ0 پێکڤە هەبونا ڤان هەردوو فاکتەرا دێ پتر زیدەکە ت مەترسیا توشبونێ ب نەخوشیێن دلی و دەمارین خوینی0

ئارمانج: مەرەم ژ ڤێ ڤەکولینێ ئەوە دیارکرنا :

ڕێژا بە ربەلاڤبونا تێکچونا کارێ ڕژینا ستوی ل دەف کەسێن توشی نەخوشیا دیاروکا ژینکار بووین0

پەیوەندی دنافبەرا هورمونێن ڕژینا ستوی و پێکهاتیێن دیاروکا ژینکار0

ڕێکێن ڤەکولینێ: سەد نەخوشا پشکداری دڤێ ڤەکولینێدا کر و ئەو نەخوش بین یێن سەرەدانا بنگەهێ دهوك یێ نەخوشیا شەکرێ کری دماوێ ڤەکولینێدا و هاتبینە دەستنیشانکرن کو نەخوشیا دیاروکا ژینکار یا هەی0 ماوێ ڤەکولینێ ژ ئێکی چریا دووێ 2010 تا 30 خزەیرانێ 2011 بو0 دە ست نیشانکرنا نە خوشیا دیاروکا ژینکار دهاتە ب جهئینان ل سەر وان بناغێن هاتینە دانان ژلایێ پروگرامێ ئەمریکی یێ کولیستیرولی (American National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III)0 پێزانین ل سەر ساخلەتێن لەشی (anthropometric measurements) هاتنە وەرگرتن و تومارکرن و نمونەیەکا بلەخرینی ژ خوینا هەر بەژداربوویەکێ هاتە وەرگرتن ژ بو شلوڤەکرنا هورمونێت ڕژینا ستوی (TSH, FT4, FT3)،ئاستێ ڕوینی دناڤ خوینێدا [کولیستیرول, ڕوینێ سیانێ (Triglyceride), ڕوینێ سەنگ بلند (HDL-Cholesterol) وڕوینێ سەنگ نزم (LDL-Cholesterol)]، ڕیژا شەکرێ د ناڤ خوینێدا (Fasting blood glucose) و هورمونێ ئەنسولین (Insulin) و هاتنە بە راوەردیکرن دگەل یێن گروپا ساخلەم (Control group) کو پێکهاتیبی ژ سەد (100) کەسێ ب سەرڤە د ساخلەم0

ئەنجام: ڕێژا بە ربەلاڤبونا تێکچونا کارێ ڕژینا ستوی پتربوو ب شێوەکێ بەرجاڤ ل دەف کەسێن توشی نەخوشیا دیاروکا ژینکار بووین بەراوردی دگەل گروپا ب سەرڤە دساخلەم0 ژ 100 نەخوشێن دیاروکا ژینکار, ئاستێ هورمونێن ڕژینا ستوی نورمال بوو (Euthyroid) لدەف 77 ٪ ژ نەخوشا, 19 ٪ لاوازیا ڕژینا ستوی یا نەکلینیکی(Subclinical Hypothyroidism) هە بوو و 4٪ لاوازیا ڕژینا ستوی یا کلینیکی (Clinical Hypothyroidism) هەبوو. ل دەف کەسێن ب سەرڤە د ساخلەم, ڕژینا ستوی نورمال بوو لدەف 96٪ ژ کەسان و 4٪ لاوازیا ڕژینا ستوی یا نەکلینیکی هەبوو0 زێدەکارکرنا ڕژینا ستوی (Hyperthyroidism) ل دەف چ کەسێن بەشداربووی نە بوو0 ڕێژا هورمونێن ڕژینا ستوی (Free T3 and Free T4) کێمتربوو ب شێوەکێ بەرجاڤ ل دەف نەخوشێن دیاروکا ژینکار ئەوێن ڕژینا ستوی لدەف وان نورمال ژ کەسێن بسەرڤە د ساخلەم (Control Group). هورمونێ TSH گرێدانەکا پوزاتیڤ (Positive Correlation) هەبوو دگەل ژمارا پێکهاتیێن دیاروکا ژینکار0 هورمونێن ڕژینا ستوی (Free T3 and Free T4) چ گریدان نەبوو دگەل ژمارا پێکهاتیێن دیاروکا ژینکار0

دەرئەنجام: د ئێتە دەرئەنجامکرن کو ڕێژا بەربەلاڤبوونا لاوازیا ڕژینا ستوی پترە ل دەف ئەو نەخوشێن دیاروکا ژینکار هەی ژ کەسێن ب سەرڤە د ساخلەم وئەڤە دبیتە ئەگرێ زێدە توشبوونێ ب نەخوشیێن دلی و دەمارێن خوینێ.

الخلاصة

معدل إنتشار قصور الغدة الدرقية لدى الأشخاص المصابين بالمتلازمة الأيضية

الخلفية: إن المتلازمة الأيضية وقصور الغدة الدرقية عاملان مستقلان من عوامل الخطورة لأمراض القلب والأوعية الدموية الناتجة من تصلب الشرايين0 إنَّ تواجد هذين العاملين معا سيزيد خطورة اللإصابة بأمراض القلب والأوعية الدموية.

الهدف: إن الغرض من هذا البحث هو معرفة:معدل إنتشار قصور الغدة الدرقية لدى الأشخاص المصابين بالمتلازمة الأيضية0

العلاقة بين هورمونات الغدة الدرقية ومكونات المتلازمة الأيضية0

طرق البحث: إشترك في هذه الدراسة مائة مريض من المرضى الذين يراجعون مركز دهوك لمرضى داء السكر والذين تم تشخيصهم بأنهم مصابون بالمتلازمة الأيضية0 أجريت هذه الدراسة في الفترة الأول من تشرين الثاني 2010 الى الثلاثين من حزيران 2011 0 تم تشخيص المتلازمة الأيضية حسب الالقواعد التي وضعت من قبل البرنامج الوطني التعليمي الأمريكي لكوليستيرول الدم–المجلد الثالث للبالغين0 تم قياس الخصائص الجسمية (Anthropometric measurements) من محيط الخصر و ضغط الدم وأخذت نماذج تحليل الدم من المرضى في حالة الصيام لقياس الهورمونات المتعلقة بالغدة الدرقية [TSH, Free Thyroxine (FT4), Free Triiodothyronine (FT3)], مستوى الدهون (Lipid Profile), الإنسولين (Insulin) وتركيز السكر(Glucose) وتم مقارنتها بنتائج المجموعة الضابطة (control group) التي تألفت من مائة أشخاص اصحاء ظاهريا0

النتائج: أظهرت هذه الدراسة أنَ إنتشار قصور الدرقية كان أكثر عند الأشخاص المصابين بالمتلازمة الأيضية بالمقارنة مع المجموعة الضابطة0 من مجموع 100 مريض بالمتلازمة الأيضية , 77٪ كانوا سوييّ الحالة الدرقية (Euthyroid), 19٪ كان لديهم قصور درقي دون السريري (Subclinical hypothyroidism) و4٪ كان لديهم قصور درقي سريري (Overt hypothyroidism)0 أما بالنسبة للمجموعة الضابطة, 96٪ كانوا سوييّ الحالة الدرقية, و4٪ كان لديهم قصور درقي دون السريري, ولكن لم يكن أي شخص من المجموعة الضابطة لديه قصور درقي سريري0 فرط الدرقية (Hyperthyroidism) لم يوجد في أي من المشاركين0 كان هورمونا الغدة الدرقية (FT4, FT3) في مرضى المتلازمة الأيضية سوييّ الحالة الدرقية أقل بشكل ملحوظ مما لدى أشخاص المجموعة الضابطة ($P < 0.01$)0 تم إيجاد علاقة إيجابية بين متوسط تركيز الهورمون المحفز للدرقية (TSH) وعدد مكونات المتلازمة الأيضية ($P < 0.05$)0 لم توجد علاقة بين هورموني الغدة الدرقية (FT3, FT4) وعدد مكونات المتلازمة الأيضية0

الإستنتاجات: يمكن الاستنتاج من هذه الدراسة بأنَ قصور الدرقية أكثر إنتشارا في الأشخاص البالغين المصابين بالمتلازمة الأيضية مقارنة مع الأشخاص البالغين غير المصابين، وهذا يجعل المصابين بالمتلازمة الأيضية أكثر عرضة للأمراض القلبية0

KNOWLEDGE OF 'PAIN ASSESSMENT AND MANAGEMENT' AMONG NURSES CARING FOR CHILDREN IN ERBIL HOSPITALS

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ABSTRACT

Background: Relieving pain has always been an essential part of the nurse caring role. Many agree that, despite all the information available to nurses, current guidelines and standards are not used consistently in the care of children's pain. A lack of knowledge about pain assessment may mean that nurses are unable to assess pain accurately and thus unable to apply their knowledge in practice.
Aim: The aim of this study was to evaluate knowledge of pain assessment and management among nurse's caring for children in Erbil City.
Methods: A descriptive cross-sectional study was conducted at 3 Hospitals in Erbil /Iraq in 2011. A purposive sample of (60) nurses who were caring for children were included. Researchers adapted the questionnaire format developed by Renee Manworren for assessment of Pediatric Nurses' knowledge regarding pain (PNKAS-Shriners revision 2002).This is consisted of two parts, Part 1 included a list of (6) items covered Socio-demographic variables and Part 2 included a list of (19) items testing nurses knowledge regarding children's pain assessment and management.
Results: Results of the study revealed that majority of nurses were females with more than five years of experience, and lacked knowledge of pain assessment and management. There was no-significant relationship between nurse's knowledge of pain assessment and management in children and their demographic characteristics.
Conclusions: Nurses caring for children in Erbil City have insufficient knowledge regarding pain assessment and management, which is related to lack of emphasis topics regarding pain in within the educational curricula.

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Key words: Assessment, non-pharmacological, pain intensity.

Pain management is an extremely important area of caring especially for children. The patient has the right to appropriate management of pain and thus the nurse is obligated to maintain current knowledge in pain assessment and management [1, 2]. The presence of pain is one of the main reasons why people seek health care, yet pain is often undertreated. Inadequate treatment has been linked to the failure of health care workers' to assess pain and to intervene appropriately [3]. O'Keeffe [4] stated that it is well established fact that children's pain is frequently poorly assessed and subsequently inadequately treated, one of the reasons for this is lack of knowledge. Mathew et al[5] carried out a survey in India to examine the knowledge and practices of nurses looking after children in the intensive care unit and they concluded that there were several lacunae in the knowledge and practice of nurses in developing countries which needed to be improved by training. Several studies have identified evidence that gaps remain in nurses' knowledge this may mean that nurses do not understand the rationale for pain-relieving interventions or that they do not know how to assess children's pain [2,6] .many authors [6,7] stated that "further research is needed in this area of nurse's knowledge and practices of pain" Although there are many studies regarding child and adolescence pain carried out around the world but there are limited studies concerning nurse's knowledge of pain" assessment and management" especially in the developing

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countries [5].There were no previous similar studies conducted in Erbil city about nurses Knowledge of pain assessment and management.

METHODS

During 2011 a descriptive study was conducted in three hospitals in Erbil(Komary Teaching Hospital; Raparin Pediatric Teaching Hospital; and Emergency Hospital) Kurdistan Region.The participants comprised a purposive sample consisted from all the nurses caring for children in the city. Official permission was obtained from the Ministry of Health –Directorate of Health – Erbil Prior to collecting the data. Permission was sought, participation was on a voluntary basis and anonymity was guaranteed.

The data was collected through a questionnaire with (26) items derived from a questionnaire developed by Renee C. B. Manworren (she is Assistant Professor at University of Connecticut School of Medicine, Pediatric Clinical Nurse Specialist and the Director of Clinical Practice Development in Texas Scottish Rite Hospital /Texas) for assessment of Pediatric Nurses' knowledge regarding pain. The questionnaire consisted of two parts: Part 1 a list of (6) items covered Socio-demographic variables. Part 2 a list of (19) items which tested nurses' knowledge regarding children's pain assessment and management. Participants responses for each knowledge question evaluated by correct or incorrect answer then scored as [(1,2)] for correct and incorrect respectively, the sum of knowledge scores for each sample obtained and after that correlated with demographic characteristics using Chi-square.

After translating the questionnaire to the regional Kurdish language content validity was tested by panel of experts and a pilot study was conducted with six nurses, questionnaire reliability was estimated by having two researchers testing the same variables (inter examiners) and validity obtained by exposing the questionnaire to expertise. The Statistical Package for Social Sciences software (SPSS, version 15) was used for data processing and statistical analysis; data was analyzed using frequencies, percentages and Chi-square.

RESULTS

(Table 1) shows that the highest percentages (40%) of the nurses were within the age group 30-39 years, and over half of them (53.3%) were female. Most of the nurses (66.6%) were working in Raparin hospital.

Table 1. Socio-Demographical Characteristics of the Nurses caring for Children in Erbil hospitals.

Variables		Frequency	%
Age	20- 29 years	22	36.7
	30-39 years	24	40.0
	40-49 years	11	18.3
	50-59 years	2	3.3
	60-69 years	1	1.7
Gender	Male	28	46.7
	Female	32	53.3
Unit of working	Raparin hospital	34	66.0
	Komary teaching hospital	11	18.3
	Emergency hospital	15	25.0

Sample No:60 nurse

(Figure 1) shows that the majority of the nurses (73.3 %) were institute graduates. (Figure 2) indicates that the majority of the nurses (38.3%) had more than five years of experience ,while (38.3%) of them had between five to ten years of experience and (11.7%) had between ten to fifteen years experience in caring for children . Concerning nurse's knowledge about pain assessment and management (Table 2 and Figure 3) reveal that the highest percentage 59.5% of nurses giving

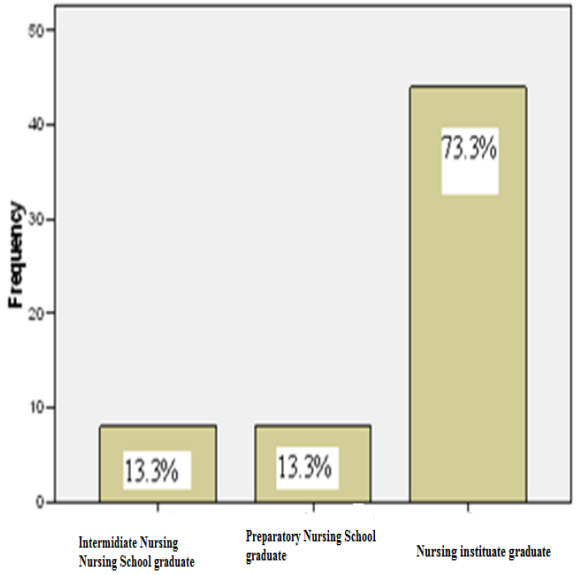

Figure 1. Educational preparation of nurses.

incorrect answers for most of pain assessment questions Table 3) shows that 56.4% of nurses giving incorrect answers for pain management questions. The relationship between nurse knowledge of pain assessment and management in children and their demographic characteristics (Unit of working, years of experience, and educational preparation) was not significant (Table 4).

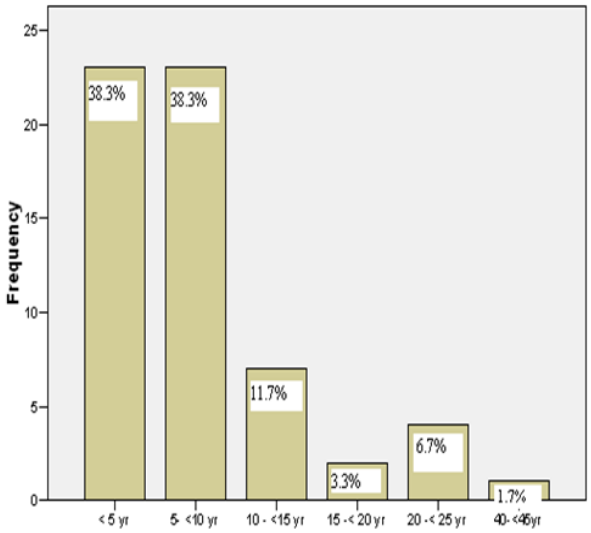

Figure 2. Nurses experience years in caring for children.

Table 2. Assessment of Nurses Knowledge about pain assessment in children.

Pain Assessment items	Correct No (%)	In correct No (%)
Observable changes in vital signs must be relied upon to verify a child's/ adolescent's statement that he has severe pain. (F)	19(31.7)	41(68.3)
Children less than 8 years cannot reliably report pain intensity and therefore, the nurse should rely on the parents' assessment of the child's pain intensity. (F)	16(26.7)	44(73.3)
Giving children/ adolescents sterile water by injection (placebo) is often a useful test to determine if the pain is real. (F)	31(51.7)	29(48.3)
Numerical pain intensity scale assessment use for child more than 9 years old. (T)	30(50)	30(50)
Wong's- baker faces pain rating scale use for patients ages 4 years through 8 years. (T)	36(60)	24(40)
If the infant/ child/ adolescent can be distracted from his pain this usually means that he is not experiencing a high level of pain. (F)	15(25)	45(75)
Infants/ children/ adolescents may sleep in spite of severe pain. (F)	23(38.3)	37(61.7)
Because of an underdeveloped neurological system, children under 2 years of age have decreased pain sensitivity and limited memory of painful experiences. (F)	23(38.3)	37(61.7)
Based on one's religious beliefs a child/ adolescent may think that pain and suffering is necessary for diminish one's guilt. (T)	26(43.3)	34(56.7)
	219(40.5)	321(59.5)

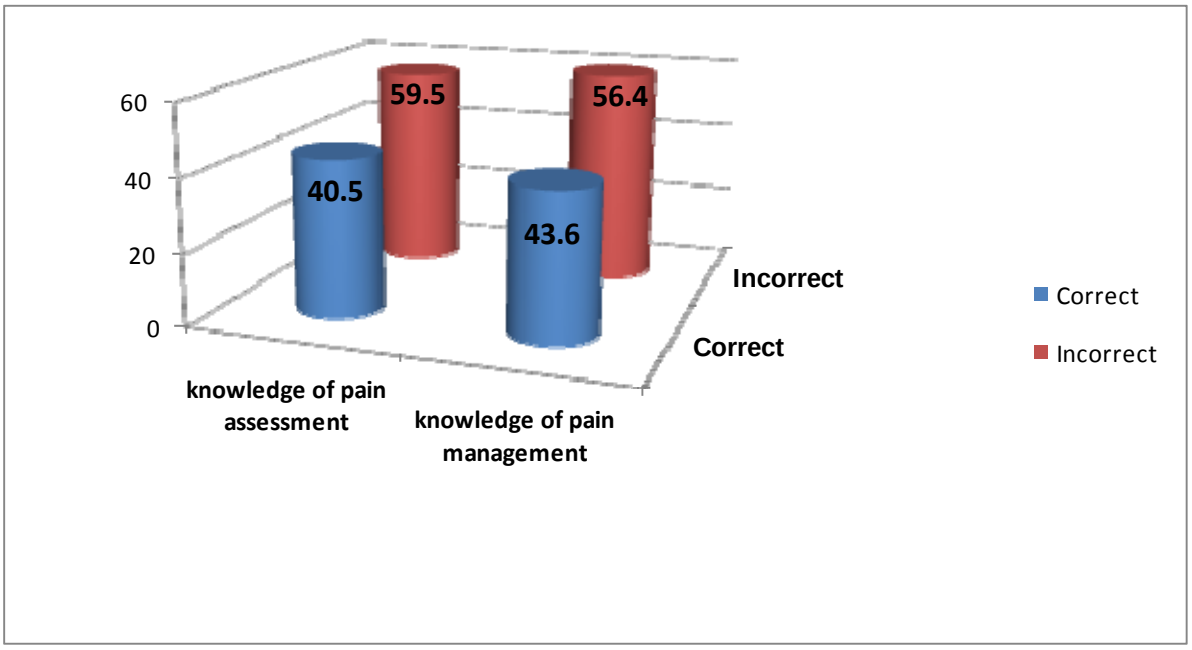

Figure 3. Nurses overall knowledge about pain assessment and management.

Table 3. Assessment of nurses knowledge about pain management in children.

Pain Management items Non-pharmacological managements	Correct No(%)	In correct No(%)
In order to be effective , heat and cold should be applied directly to the painful area.(T)	36(60)	24(40)
The child/ adolescent should be advised to use non-drug techniques (Film cartoon , child story and toy) alone rather than concurrently with pain medications.(F)	18(30)	32(53)
Parents should not be present during painful procedures.(F)	35(58.3)	25(41.7)
Non-drug interventions (e.g. heat, music, imagery, etc.) are very effective for mild-moderate pain control but are rarely helpful for more severe pain. (F)	17(28.3)	43(71.7)
Pharmacological managements		
Adolescents with a history of substance abuse should not be given opioids for pain because they are at high risk for repeated addiction.(F)	22(36.7)	38(63.3)
Respiratory depression rarely occurs in children/ adolescents who have been receiving opioids over a period of months. (T)	38(63.3)	22(36.7)
The usual duration of analgesia of Morphine IV is 4-5 hours.(F)	22(36.7)	38(63.3)
Sedatives are appropriate medications for the relief of pain during painful procedures.(F)	17(28.3)	43(71.7)
Young infants, less than 6 months of age, cannot tolerate opioids for pain relief.(F)	23(38.3)	37(61.7)
Children who will require repeated painful procedures should receive maximum treatment for the pain and anxiety of the first procedure to minimize the development of anticipatory anxiety before subsequent procedures.(T)	33(55)	27(45)
	(261) 43.6	(339)56.4

T True , F* False*

Table. 4 Correlation ship's between Nurses Knowledge and Some Related Variables.

Crosstab Causes Correlation ship's	Chi- Square	Approx. Sig	Evaluation
Unit of working* Nurses Knowledge	**24.798**	**.417**	**NS***
Number of experience years in pediatric* Nurses Knowledge	**34.916**	**.698**	**NS**
Educational preparation* Nurses Knowledge	**13.828**	**.611**	**NS**

NS non-significant*
NS: at P –Value > 0.05 S : at P –Value < 0.05 HS: at P –Value < 0.01

DISCUSSION

In this study, the nurses' responses indicated that there was deficiency in knowledge in many areas, including: pain assessment, the pharmacology of analgesic drugs, the use of analgesic drugs, and non-pharmacological methods. Few nurses knew that they could not rely upon observable changes in vital signs when verifying a child's/adolescent's statement that they have severe pain, Just under three-quarters of them thought that children less than 8 years could not report pain intensity, therefore, they believed the nurse should rely on the parents' assessment of the child's pain intensity. Three quarters of nurses believed that if the infant/ child/ adolescent could be distracted from his pain this usually meant that they are not experiencing a high level of pain. Over half of the participants thought that infants/ children/ adolescents would sleep in spite of severe pain. A high number thought that children under 2 years of age had decreased pain sensitivity and limited memory of painful experiences because of an underdeveloped neurological system (Table 2). Regarding Non-pharmacological managements few nurses had knowledge about non-drug techniques (Film cartoon, child story and toy) in pain relieving. This is in contrast to the findings of Mathew et al [5] who found that restraint and distraction were the common modalities employed to facilitate painful procedures.

Regarding pharmacological managements most of nurses thought that adolescents with a history of substance abuse should not be given opioids for pain because they would be at high risk for repeated addiction, a highest percentage thought that the usual duration of analgesia of Morphine IV was 4-5 hours. Many were not aware that young infants, less than 6 months of age, could tolerate opioids for pain relief (Table 3). This concurs with McCaffery and Ferrel who claimed that health care professionals had inaccurate knowledge of commonly used analgesic agents and many had fears about the incidence of opioid addiction[8].In common with other [9, 10, 11, 12,13, 2] this study confirmed that the majority of nurses had deficient knowledge concerning children pain assessment and management. Researchers found that there was little correlation between nurse's knowledge of pain assessment and management in children and their demographic characteristics (Unit of working, years of experience, educational preparation) (Table 4). This could be because of the lack on emphasis on topics regarding pain in within the educational curricula and in service education.

CONCLUSIONS AND RECOMMENDATIONS

Nurse caring for children in Erbil City had deficits in their knowledge regarding pain assessment and management. So the researchers recommended enriching the nursing curricula with pain topics and developing an educational program and submitting of seminars for nurses to increase their knowledge about pain assessment and management.

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پوختە

زانیاری پە رستاران دەربارەی هە لسەنگاندن وچارەسەری ئازاری منداڵان لە نەخۆشخانە کانی هەولێر

پێشەکی: لابردنی ئازار هەموو جاری لە رولە بنەرەتیەکانی چارەسەری پە رستاریە ,زوربەی تویژەران هاودەنگن لەوەی جگە لەو هەموو زانیاریە کە لەبەر دەستی پە رستاران داهە یە , بەلام نەخشەریگاو ستانداردەرکان بەتەواوەتی بەکار ناهینن بو چارەسەری ئازاری منداڵان ,کەمی زانیاری پە رستاران دەربارەی هە لسەنگاندن بو ئازاری منداڵان لەوانەیە ئەمە وا بگەینیت کە پە رستاران نەتوانن بەتەواوەتی هە لسەنگاندنی ئازار بکەن وە ئەمەش وا دەکات زانیاریە کانیان نەتوانن بەکار بهینن لە بواری کرداری.

ئامانج: ئامانجی ئەم توێژینەوەیە بریتیە لە هەڵسەنگاندنی زانیاری پە رستاران دەربارەی هە لسەنگاندن وچارەسەری ئازاری منداڵان لە شاری هەولێر

جوری توێژینەوە: توێژینەوەیەکی وەسفی لە (3)نەخۆشخانەکانی شاری هەولێر – عیراق (2011)ئەنجام درا, توێژینەوەکە لە وەرگرتنی(60) پە رستاران کە چارەسەری منداڵان دەکەن ,لیستی پرسیارەکان وەرگیراو لە (Renee Manworren, (2002 (PNKAS-Shriners revision) کە ئامادەکراوە بو هەڵسەنگاندنی زانیاری پە رستاری بو ئازاری منداڵان دوای هەندیك گۆڕانکاری کراوە لەلایەن توێژەر, پرسیارەکان پێکهاتووە لە دوو بەش: بەشی یەکەم بریتیە لە (6) پرسیار دەربارەی باری دیموگرافی پە رستاران, بەشی دووەم بریتیە لە (19) پرسیاری دەربارەی زانیاری هە لسەنگاندن وچارەسەری ئازاری منداڵان .

ئەنجام: ئەنجامی ئەم توێژینەوەیە ئەوە دەردەخات کە زۆربەی پەرستاران میینەن و زیاتر لە(5)سال راژەیان هەیە وە کەمی زانیاری دەربارەی هە لسەنگاندن وچارەسەری ئازاری منداڵانن . ،وەئەوەش دەردەخات کە هیچ پەیوەندیەکی گرنگ لەنێوان زانیاری پە رستاران دەربارەی هە لسەنگاندن وچارەسەری ئازاری منداڵان و باری دیموگرافی پە رستاران نیە.

دەرکەوتن: چارەسەری پە رستاری بو منداڵان لە شاری هەلیر کەمی زانیاریان هەیە دەربارەی هە لسەنگاندن وچارەسەری ئازاری منداڵان ,کە ئەمەش دەگەریتەوە بو نەبوونی بابەتی گرنگ دەربارەی ئازار لە پروگرامی خویندنی پەر ستاری.

الخلاصة

معارف تقييم ومعالجة الالم لدى الممرضات اللواتي يعتنون بالاطفال في مشتشفيات اربيل

خلفية البحث: ان رعاية المريض والتقليل من الالم هو جزء اساسي في الرعاية التمريضة والكثيرون يوافقن على انه بالرغم من وجود المعلومات الكافية والدليل للرعاية التمريضية لتخفيف ومعالجة الالم لدى الاطفال لكن الممرضات لا يطبقونها دائما.ان النقص في المعلومات والمعارف المتعلقة بالالم يؤدي الى عدم تمكن الممرضه من تقييم الالم وبالتالي عدم معالجه المريض.

الاهداف: ان الهدف من البحث هوتقييم المعارف الخاصة بتقييم ومعالجة الالم لدى الممرضات اللواتي يعتنين بالاطفال في مستشفيات اربيل.

طرق البحث هذه دراسة وصفيه اجريت في ثلاثة من مستشفيات في مدينة اربيل (مستشفى الجمهوري التعليمي, مستشفى رابرين للاطفال ومستشفى الطوارئ) خلال عام 2011 . كانت عينة البحث عبارة عن عينة غرضية عددها ستون ممرضة وممرض ممن يقدمون الرعاية التمريضية للاطفال وذلك بعد استحصال الموافقات الاصولية لاجراء البحث. تم

استعمال المعيار(الاستبيان) الذي بناه (رينيه مانورن) الخاص بتقييم معارف الألم لدى الممرضات والذي يتكون من جزئيين لجمع المعلومات. الجزء الأول متكون من ستة فقرات حول المعلومات الديموغرافية، والجزء الثاني متكون من تسعة عشر فقرة حول معارف تقييم ومعالجة الألم عند الأطفال.

النتائج: لقد اظهرت النتائج بان اغلبية الممرضات من الاناث ولهم خدمة اكثر من خمسة سنوات و معارفهم المتعلقة بتقييم ومعالجة الالم عند الاطفال ضعيفة .

الاستنتاجات: استنتج الباحث بان الممرضات اللواتي يعتنين بالاطفال في مدينة اربيل ليس لديهم معلومات كافية حول تقييم ومعالجة الالم عند الاطفال والذي يرجع الى قلة المفردات عن الالم في المناهج الدراسية للممرضات. كما اشار الباحث الى عدم وجود علاقة معنوية ذات دلالة احصائية بين معارف تقييم ومعالجة الالم والمتغيرات الديموغرافية الاخرى للممرضات وذلك يعود لقلة احتواء المنهاج الدراسي للممرضات على المواضيع الخاصة بمعالجة الالم لدى الاطفال .

THE EFFECT OF LOXOPROFEN SODIUM ON SALIVARY CONCENTRATION OF SUBSTANCE P FOLLOWING SURGICAL REMOVAL OF IMPACTED MANDIBULAR THIRD MOLAR

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ABSTRACT

Aim: To evaluate the efficacy of loxoprofen sodium on substance P salivary concentration after the surgical removal of mandibular wisdom tooth.
Methods: Surgical removal of mandibular third molar was performed in two groups of individuals, total of fifty patients were anticipated in this study. All were medically fit, their average of age 24.48± 5.27 (ranged 17-38); each group consisted of twenty five patients. Group A: Preoperative 120 mg loxoprofen sodium oral capsule 30 minutes prior surgical operation; group b: placebo or control group where 120 mg glucose oral capsule 30 minutes prior surgical operation was applied. all patients received post operatively amoxicillin capsules 500 mg t.i.d, metronidazole tablet 500mg t.i.d, and supplementary paracetamol 500 mg tablet as required. Salivary concentration of substance P was measured by ELISA. Pain was assessed by verbal pain scale, and duration of postoperative analgesia which recorded between groups.
Results: Postoperative salivary concentrations of substance P, verbal pain scale, and duration of postoperative analgesia in drug group was significantly lower than those in control group ($P<0.05$).
Conclusions: Preoperative loxoprofen sodium reduced postoperative concentrations of substance P in saliva. Substance P salivary levels significantly increased at first day following surgical removal of impacted mandibular third molar and it's seem to be correlated with postoperative pain.

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Key words: Substance P, Loxoprofen, Impacted mandibular third molar and Postoperative pain.

The removal of impacted teeth is one of the most common procedures performed by oral and maxillofacial surgeons. The dental impaction pain model has become one of the primary models used in developing analgesic drugs, as it provides a readily available healthy population and a relatively uniform surgical procedure confined to one area of the body [1].

Substance P (SP) consists of 11 amino acids and is a prototypic neuropeptide for more than 50 neuroactive molecules, which include CGRP, neuropeptide Y, and endothelin [2,3]. Originally it was isolated from intestinal extracts, it's ability to produce hypotension and non propulsive contractions of isolated intestine were first reported in the 1930 [4-6]. The amino acid sequence was not established until 1971 [7]. It is of considerable scientific and therapeutic interest because of its location in discrete cell groups in the gastrointestinal system, spinal cord, and brain [8]. SP is typically co-localized with other peptides and/or amine neurotransmitters, it is released from neurons and acts as a neurotransmitter or a neuromodulator.

In the periphery, SP is a neurotransmitter in primary sensory neurons with cell bodies in the dorsal root ganglia and cranial sensory ganglia. These neuron s not only transmit sensory information from the periphery to the central nervous system, but are also important for the local release of SP, leading to neurogenic inflammation, a condition characterized by vasodilatation

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and increased vascular permeability [9]. Peripheral sensitization can be with different stimuli, thermal, tactile, mechanical, and chemical, and their first relay is in the dorsal horn. In clinical situation this noxious stimuli is prolonged resulting in tissue damage and inflammation. This leads to release of a 'soup' of inflammatory mediators such as SP which in turn acts to sensitize high threshold nociceptors. As the low threshold stimuli, which would not normally cause pain, are now perceived as painful [10].

In most studies, SP was more concentrated in saliva than in serum or plasma. Therefore, it appears that SP does not merely "leak" from serum into saliva but actively enters saliva by an unknown mechanism [11]. Almost all pathological conditions that affect oral tissues, as well as orthodontic or dental care procedures, increase the production and release of SP [12].

Loxoprofen sodium (loxoprofen), a non steroidal anti-inflammatory drug (NSAID), is a nonselective cyclooxygenase (COX) inhibitor and works by reducing the synthesis of prostaglandin from arachidonic acid. It's of the phenylproionic acid group. Loxoprofen sodium is clinically used as an anti-inflammatory, analgesic and antipyretic agent. The chemical structure is sodium 2-[4-(2-oxocyclopentan-l-methyl) phenyl] propionate dihydrate which was first synthesized in the Central Research Laboratory of Sankyo Co. Ltd., Japan. It is characterized in potent analgesic action compared to the anti-inflammatory and antipyretic actions [13]. The analgesic activity of loxoprofen in animal study is approximately 20 times greater than that of either indomethacin or naproxen and approximately 10 times greater than that of ketoprofen and exhibits relatively weak gastrointestinal ulcerogenicity [14]. Loxoprofen is effective for relief pain after tooth extraction. Loxoprofen is a prodrug. It is quickly converted to its active transalcohol following oral administration, and reaches its peak plasma concentration within 30 to 50 minutes [15].

Aim of study: To evaluate the efficacy of loxoprofen sodium on substance P salivary concentration after the surgical removal of mandibular wisdom tooth.

METHODS

Study design and samples

A double blind randomized study was performed at the Department of Oral and Maxillofacial Surgery, College of Dentistry, University of Mosul, and included fifty seven patients who required surgical removal of a single impacted mandibular third molar under local anesthesia (2%lidocaine and 1/80000 of adrenaline in 1.8 ml). Mean age of patients (25 males and 25 females) was 24.48± 5.27 years with a range of (17-38 years). The patients were divided into two groups: Group A (control) twenty five patients and Group B (loxoprofen) twenty five patients.

Patient's selection

All patients required the surgical removal of mandibular third molar required bone removal, that were free of pericoronitis and infection at the time of surgical procedure, no history of allergy or hypersensitivity to the loxoprofen, no history of compromised medical status, no recent use of anti-inflammatory and antibiotic drugs, non-pregnant or lactating females, non-smoking, non-alcoholic and agreed to participate in this study and signed the consent form. The study was approved by the local academic committee according to relevant guidelines.

Patients grouping

Double blind randomized study was performed. Patients were divided into 2 groups; Group A: (25) patients received code A (preoperative oral capsule of 120mg loxoprofen 30 minutes prior to surgery). Group B: (25) patients received

code B (preoperative oral capsule of 120mg glucose 30 minutes prior to surgery).

Surgical procedure

A standardized surgical procedure was performed on all patients by the same right-handed operator in the same operating room and under similar conditions. A small gauze pack was then applied on the surgical site and the usual post extraction instructions were given to the patient. Duration of surgery in minutes (from beginning of initial incision to the last suture) was recorded.

Postoperative medications

All patients in the study (including those in control), routinely received amoxicillin (oral 500 mg every 8 hours) for 5 days following surgery, metronidazole tablets (500 mg every 8 hours) and paracetamol tablets (oral 500 mg on need). In addition, a 0.2% chlorhexidine mouth rinse was prescribed twice daily to be started the day after surgery for 5 days.

Postoperative pain assessment

Pain was objectively measured at 24 hours after the end of operation.

Verbal pain scale (VPS): The scale consists of: 0: no pain; 1: mild pain; 2: moderate pain; 3: severe pain; and 4: very severe pain (16).

Duration of analgesia (DA): Patients were instructed to record the first time for the need of analgesia (Paracetamol tablet 500 mg) after surgical operation (time from the end of the surgery until the intake of rescue medication became necessary for the patient).

Saliva collection and substance P measurement

Whole unstimulated saliva was collected at 30 minutes before start of operation and 24 hours after the end of operation, and at least 2 hours after any food intake to exclude the food stimulatory effect on salivary secretions. SP immunoassay ELISA kits (R&D Systems, Inc., USA) were used to determine the concentration of SP in the whole-saliva samples. The assay was performed according to the manufacturer's instructions and the results referred to a calibration curve expressed in pg /ml. Samples were assayed in duplicate.

Statistical Analysis

Data were incrementally entered over the course of study into an electronic sheet (Excel, Microsoft inc, Windows 2007) and analyzed using statistical package for social sciences (SPSS) program version 19.0. Analysis included descriptive statistics (frequency and percentages for non–parametric data, and mean and standard deviation for parametric ones); and analytical statistics (Mann–Whitney Test for non–parametric data and independent sample Student's t–test for parametric ones was applied among the data of pre and post values of the parameter). Differences between groups were considered statistically significant when $p < 0.05$ level.

RESULTS

The results obtained in this study were represented as standard descriptive statistic of mean ± standard deviation for the measured values before and after the surgical operation. There were no missing data and all patients included in the study attended the follow up visit.

Verbal pain scale of study groups

The results of Verbal pain scale (VPS) were measured at 24 hours after the surgery. The results and statistics obtained for VPS for control group were mild (8%), moderate (48%) and sever (44%) and for group B were mild (52%), moderate (48%) and sever (0%). There was significant difference (at P=0.001) of VPS of study groups as showed in(Table 1).

Table 1. Descriptive statistics and Mann-Whitney U Test of VPS of study groups.

Groups	VPS	Frequency	Percent (%)	Mann Whitney U	Z-value	P-value
Control	Mild	2	8	109	-4.277	0.001*
	Moderate	12	48			
	Sever	11	44			
	Total	25	100			
Loxoprofen	Mild	13	52			
	Moderate	12	48			
	Sever	0	0			
	Total	25	100			

** Significant difference existed between groups at p=0.001 levels.*

Duration of analgesia of study groups

The results of duration of analgesia (DA) (hours) were measured during 24 hours after the surgery. The results and statistics obtained for DA for group A and B were 3.26± 1.595 and 6.46±2.692 hours respectively. There was significant difference (at p=0.001) of DA of study groups as showed in (Table 2).

Table 2. Descriptive statistics and independent samples T-Test of duration of analgesia of study groups.

Groups	Number	Mean	S.D	S.E mean	t- value	df	P-value
Control	25	3.26	1.595	0.319	-5.113	48	0.001*
Loxoprofen	25	6.46	2.692	0.538			

** Significant difference existed between groups at p=0.001 levels.*

Pre- and post-operative SP salivary concentration of study groups

The results of SP salivary concentration were measured 30 minutes before and at 24 hours after the surgery. The results and statistics obtained for SP salivary concentration for group A pre and postoperatively were 46.44±5.082 and 61.68±8.097 pg/ml respectively and for group B the pre and postoperatively were 47.95±5.283 and 56.82±7.280 pg/ml respectively. There was no significant difference (at p=0.308) of pre-operative SP salivary concentration while there was significant difference (at p=0.036) of post-operative SP salivary concentration of study groups as showed in (Table 3).

Table 3. Descriptive statistics and independent samples T Test of Pre and Postoperative SP salivary concentration (pg/ml) of study groups.

Time	Groups	Number	Mean	S.D	S.E mean	t-value	df	P-value
Pre	Control	25	46.44	5.028	1.005	-1.031	48	0.308
	Loxoprofen	25	47.95	5.283	1.056			
Post	Control	25	61.68	8.097	1.619	2.234	48	0.036*
	Loxoprofen	25	56.82	7.280	1.456			

** Significant difference existed between groups at p<0.05 levels.*

DISCUSSION

Preoperative analgesia is involves delivery of analgesic therapy that proceeds, adequately blocks, and outlasts the nociceptive stimuli that accompany tissue injury. The aim is to prevent the peripheral and central sensitization that occurs in response to painful stimuli, while leaving physiological pain responses intact. Such an effect reduces primary and secondary

hyperalgesia, allodynia and the receptive field changes of dorsal horn cells. Opioids, NSAIDs, local anesthetics, alpha-2 agonists and NMDA receptor antagonists were considered the main agents in the preemptive analgesic [17].

Pain management can be established by the administration of analgesia during either preoperative administration of some analgesic to reduce the onset of post operative pain [18]. Patient self–report is the most accurate and reliable indicator of the existence and intensity of pain and any resultant distress [19]. NSAIDS and paracetamol are agents used in postoperative pain management. Many studies have reported oral paracetamol as an agent that is effective and well tolerated in different surgical procedures [20].

In the present study, VPS and DA have been implicated to describe the intensity of pain. The data demonstrated that the preoperative loxoprofen was more effective in reducing VPS than placebo as analgesia. The longer DA in loxoprofen group can be explained by the fact that the analgesic effect of loxoprofen before the nociceptive stimuli was effective.

SP is an important nerve transmitter of pain. Its concentration is directly proportional to the injury stimulation. Results of determination of sensory neuropeptide levels in plasma and spinal fluid have suggested that measuring these peptides would be more efficient if performed closer to the effectors [21]. Saliva is a fluid close to the receptors as plasma or lumbar spinal fluid [22]. Thus, we proposed that saliva could represent a fluid particularly suitable for analysis of release of neurotransmitters following surgical removal of mandibular third molar. Preoperative loxoprofen sodium reduced postoperative concentrations of SP in saliva. SP salivary levels significantly increased at first day following surgical removal of impacted mandibular third molar and it's seem to be correlated with postoperative pain. Therefore, SP can objectively reflect the degree of pain [23].

CONCLUSIONS

Preoperative loxoprofen sodium reduced postoperative concentrations of substance P in saliva. Substance P salivary levels significantly increased at first day following surgical removal of impacted mandibular third molar.

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پوخته

کارتێکرنا دەرمانلێ لوکسوبروفین صودیوم ل سەر چریا کەرستا (پ) د خوزیا مروڤی دا پشتی دەرهێنانا ددانێ ئاقلێ د شەوکا بنیدا

پێشەکی و ئارمانج: ئارمانجا ڤێ ڤەکولینێ هەلسەنگاندنا ریژا کارتێکرنا لوکسوبروفین صودیوم ی ل سەر چریا کەرەستا (پ) د خوزیێ دا پشتی ینانەدەرا ددانێ ئاقلی د شەوکا بنی دا ب ریکا نشتەرگەریێ.

ریکێن ڤەکولینێ: ئینانا دەرا ددانێ ئاقلی د شەوکا بنیدا ب ریکا نشتەرگەریێ هاتە ئەنجامدان بو (50) نەخوشێن هاتینە دابەشکرن ل سەر دوو گروپان پێکهاتی ژ (25) نەخوشان بو هەر ئێکێ ئەوێن ژیێن وان ژ 17–38 سال. گروپێ ئێکێ کەپسولەکا 120 ملیگرامی ژ لوکسوپروفینا صودیومی هاتە دان ب ریکا دەڤی بەری ئینانەدەرا ددانی ب 30 خولەکان، گروپێ دووی کەپسولەکا 120 ملیگرامی ژ گلوکوزی هاتە دان ب ریکا دەڤی بەری ئینانەدەرا ددانی ب 30 خولەکان. پشتی نشتەرگەریێ بو هەردوو گروپان (500) ملیگرامێن دەرمانێ (ئەموکسیسیلین) و(500) ملیگرامێن دەرمانێ میترونیدازول سێ گەرا د روژێدا هاتە دان دگەل (500) ملیگرامێن پاراسیتولێ ل دویف پێدڤیێ. ریژا کەرستێ (پ) هاتە تاقیکرن ب ریکا ئامیرێ (ئیلایزەر) گرانیا ئێشێ هاتە تاقیکرن ب ریکا گوتنا نەخوشی.

ئەنجام: ریژا کەرستێ (پ) د خوزیێ دا پشتی کارێ نشتەرگەری و دەربرینا گرانیا ئێشێ ب ریکا گوتنا نەخوشی ل گروپێ ئێکێ کێمتر بوو ژ گروپێ دووێ.

دەر ئەنجام: ب کارئینانا لوکسوپروفین صودیوم بەری نشتەرگەریێ دێ چریا کەرستا کێم کەت.

چریا کەرستا(پ) دخوزیێ دا زێدە دبیت د روژا ئێکێ دا پشتی کارتێکرنا نشتەرگەریێ لسەر ددانێ ئاقلی ئەوی د شەوکا بنیدا.

الخلاصة

تأثير عقار لوكسوبروفين الصوديوم على التركيز اللعابي للمادة بي بعد القلع الجراحي لسن العقل المطمور في الفك السفلي

الهدف من الجراحة: ان الهدف من الدراسة لتقييم فعالية لوكسوبروفين الصوديوم على التركيز اللعابي للمادة بي بعد القلع الجراحي لسن العقل المطمور في الفك السفلي.

المواد وطرائق العمل: تم تنفيذ القلع الجراحي لسن العقل المطمور في الفك السفلي لخمسين مريض, قسموا الى مجموعتين من الأفراد وتألفت كل مجموعة من خمسة وعشرين مريضا جميعهم لائقين طبيا, وكان متوسط أعمارهم 5.27 ±24.48 (تراوح بين 17الى 38) . المجموعة الأولى: تم اعطاها كبسولة تحتوي على 120 ملغ من لوكسوبروفين الصوديوم عن طريق الفم, قبل القلع الجراحي ب 30 دقيقة. المجموعة الثانية: تم اعطاها كبسولة تحتوي على 120 ملغ من الكلوكوز عن طريق الفم, قبل القلع الجراحي ب 30 دقيقة. تم اعطأء جميع المرضى بعد الجراحة أموكسيسيلين كبسول 500 ملغم ثلاث مرات يوميا، ميترونيدازول قرص 500 ملغم ثلاث مرات يوميا والتكميلية قرص باراسيتامول 500 ملغ عند الحاجة. تم قياس السبستانس بي بإستخدام جهاز الإيلايزا. شدة الألم تم قياسها باستخدام طرقة "المقياس الكلامي للألم" و "فترة تسكين الألم بعد العملية".

النتائج: كان التركيز اللعابي للمادة بي بعد العملية الجراحية, المقياس الكلامي للألم و فترة تسكين الألم بعد العملية في المجموعة الأولى اقل من المجموعة الثانية.

الأستنتاج: تناول اللوكسوبروفين الصوديوم قبل العملية الجراحية يقلل التركيز اللعابي للمادة بي. التركيز اللعابي للسبستانس بي يزداد في اليوم الأول بعد القلع الجراحي لسن العقل المطمور في الفك السفلي

GALL STONES: A CLINICO-PATHOLOGICAL STUDY OF (275) CASES IN DUHOK CITY

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ABSTRACT

Introduction: Gall stones disease is one of the most common problems affecting the digestive tract. Autopsy reports have shown a prevalence of gallstone from (11%) to (35%). The prevalence of gallstones is related to many factors, including age, gender and ethnic background. Cholelithiasis produces diverse histopathological changes in gallbladder mucosa namely acute inflammation, chronic inflammation, glandular hyperplasia, granulomatous inflammation, cholesterolosis, dysplasia, and carcinoma.
The aims of this study were to find out the age and gender distribution of the gall stones in Duhok City, the anatomical anomalies in the biliary system and main pathologic changes in the gallbladder in association with gallstones.
Methods: This study included (275) patients; (51) males and (224) females, all of them had cholecystectomy for cholelithiasis. The stones were analyzed chemically and the gallbladders were studied histopathologically.
Results: This study showed that females were affected more than males (4.3:1) ratio; the mostly affected age group was the fourth decade of life (30-39) years. Mixed stones were the commonest (60.3%) followed by cholesterol stones (25%). Anomalies of the biliary system were detected in (16) patients and include cystic duct variations (4.3%) and cystic artery variations (1.4%). The commonest histopathological finding was chronic cholecystitis (68.3%) and malignancies constituted only (1%).
Discussion: The present work is consistent with the work of many other researchers regarding the age and gender distribution of gallstones and also regarding the effects of gallstones on the gallbladder. But, regarding the type of stones whether cholesterol, mixed or bilirubinate stones, there are some dissimilarity with the results of some workers.
Conclusions which can be made from this study are that; gallstones are more common in women than men, the fourth decade of life is the peak incidence, the commonest pathology is chronic cholecystitis, malignancies are rare and anatomical anomalies are very important surgically.

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Key words: Gallbladder, Gallstones, Cholecystitis.

Gallstones disease is one of the most common problems affecting the digestive tract. Autopsy reports have shown a prevalence of gallstone from (11%) to (35%). The prevalence of gallstones is related to many factors, including age, gender and ethnic background. Certain conditions predispose to the development of gallstones. Obesity, pregnancy, dietary factors, Crohn's disease, terminal ileal resection, gastric surgery, hereditary spherocytosis, sickle cell disease and thalassemia are all associated with an increased risk of developing gallstones. Women are three times more likely to develop gallstones than men and first degree relatives of patients with gallstones have a twofold greater prevalence.[1]

The incidence of cholelithiasis in the USA is about (10%) with about (800,000) new cases seen annually, (70%) of affected women acquiring them by age of (30) years and (70%) of men by age of (60) years. [2] A study done in Italy reported that

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overall prevalence was (11%), (6.7%) in men and (14.6%) in women ranging from (18) to (65) years of age. [3]

Cholesterol and bile pigments are the two principal constituents of gall stones. In addition Calcium Carbonate, Phosphate and Palmate are present in variable amounts. Pure cholesterol and pure pigment stone do occur, but most stones are mixed. Predominantly cholesterol stones account for (75%) of all gallstones in the west. They are single or multiple, hard and usually layered on cross-section. Pigment stones are most common in Asia. They are usually black or brown in color, brown stones crumble when squashed. About (10%) of stones contain enough Calcium to be radio-opaque. [1]

Diseases in the gall bladder affect only a small minority of people with gallstones. It presents with a variety of clinical syndromes, of which the most common are chronic cholecystitis, which appears slowly overtime, biliary colic and acute cholecystitis, both of which develop rapidly. [4]

Acute cholecystitis is secondary to gallstones in (90%) to (95%) of cases. In less then (1%) of acute cholecystitis, the cause is a tumor obstructing the cystic duct. [1]

Histologically, the inflammatory reactions are not distinctive consist of the usual patterns of acute inflammation (i.e. edema, leucocytic infiltration, vascular congestion, frank abscess formation, or gangrenous necrosis). In chronic cholecystitis, the changes are extremely variable and sometimes minimal .The mere presence of stones within the gallbladder, even in the absence of acute inflammation, is often takes as sufficient justification for the diagnosis. The gallbladder may be contracted, of normal size, or enlarged. Mucosal ulcerations are infrequent; the submucosa and subserosa are often thickened from fibrosis. In the absence of superimposed acute cholecystitis, mural lymphocytes are the only sentinels of inflammation.[5]

Chronic cholecystitis is generally associated with gallstone and may follow acute cholecystitis or develop insidiously. Features of acute cholecystitis may be superimposed on it. The gallbladder is thickened and fibrotic, muscle fibers are hypertrophied .The mucosal epithelium may be atrophic or hyperplastic, sometimes forming diverticula which can reach the serosal surface, these diverticula are often called (Rokitansky-Aschoff sinuses). The presence of cholesterol and bile in damaged diverticula stimulates a xanthogranulomatous response, with large numbers of foamy histiocytes and multinucleated foreign body giant cells containing cholesterol crystals. Severe chronic cholecystitis often causes fibrosis of the gallbladder bed so that inflamed organ is firmly adherent to the liver and difficult to remove. [6]

All tumors of gallbladder are rare, but adenocarcinoma is the most frequent. More than three-quarters of cases have gallstones. Occasionally squamous carcinoma has been described, presumably arising from metaplastic squamous epithelium. Gallstones are invariably found in these cases. [6,7]

The aims of this study:

-To find out the age and gender distribution of the gall stones in Duhok City.

-To find out the anatomical anomalies in the biliary system.

-To find out the main pathologic changes in the gallbladder in association with gallstones.

METHODS

This study includes (275) cases, (51) male and (224) female. All of them operated on for cholecystectomies, whom were admitted to private and public hospitals in Duhok city for cholelithiasis.

Preoperatively, history was taken and clinical examination was performed. Important and relevant laboratory and

ultrasonographic investigations were done also.

Gallbladders were examined grossly, then opened and stones were removed, examined grossly and then submitted for chemical analysis. Three sections from each gallbladder were taken, from the fundus, body and neck. These sections were processed automatically, embedded in paraffin wax, blocked; cut by a microtome into (4) micron-thick sections, mounted on glass slides and then stained by hematoxylin and eosin stains. [8]

Patients were classified according to age, gender, number of stones and the result of chemical analysis. Regarding the histopathologic examination, the findings were categorized in this way:

1.**No significant histopathological findings**.

2.**Inflammation:**

a.Acute cholecystitis.

b.Acute on chronic cholecystitis.

c.Chronic cholecystitis.

3.Malignancy.

The results were expressed in frequencies and percentages.

Anatomical variations were recorded according to the operative findings of the surgeon.

RESULTS

This study included (275) patients; (51) were males (19%) and (224) were females (81%) (Figure 1).

Of the included patients; (77) were in the 4th decade of life (30-39 year) (28%) and only (5) patients were below twenty years (1.8%) (Figure 2). Two of them had hereditary haemolytic anaemia.

The stones were multiple in (206) patients (75%) (Figure 3) and (166) patients (60.3%) had mixed stones (Figure 4).

Anatomic anomalies were detected in (16) patient; (12) of them have cystic duct variations and (4) have cystic artery variations (Figure 5).

The commonest histopathological diagnosis was chronic cholecystitis (188) patients (68.3%) (Figures 6 and 7).

(Figure 8) is a microphotograph of acute haemorrhagic cholecystitis, (Figure 9) shows cholesterolosis and(Figure 10) shows intestinal metaplasia. (Table 1) summarizes the histopathological diagnoses of the included (275) patients. The most frequent histopathological findings were mucosal ulceration and Rokitansky Aschoff sinuses which have been seen in (92) patients (33.4%) and (49) patients (21.8%) respectively (Table 2). Only (3) patients (1%) had gallbladder carcinoma. The latter group include two males and one female; two of them had adenocarcinoma (Figure 11) and one had squamous cell carcinoma.

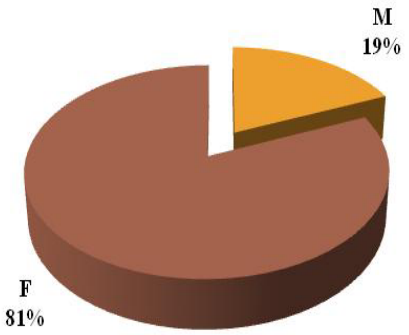

Figure 1. Gender distribution
M: Male F: Female

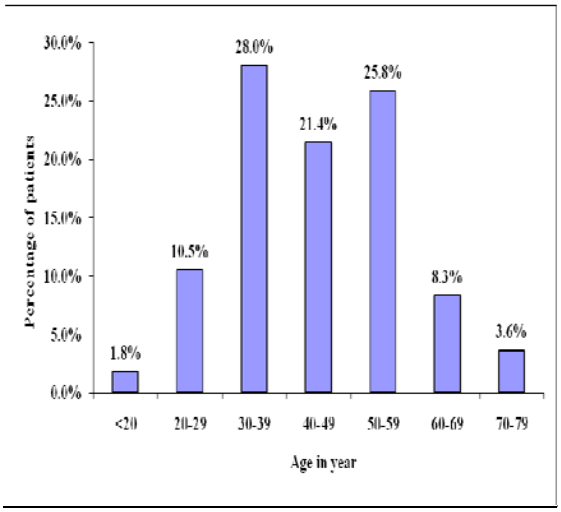

Figure 2. Age distribution and percentage of the patients

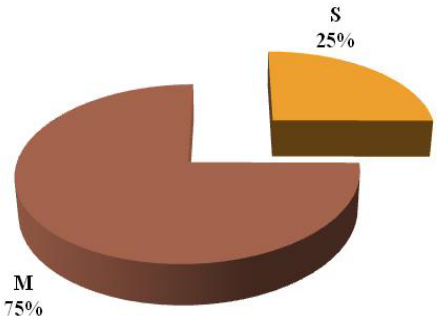

Figure 3. Number of gallstones S: Single M: Multiple

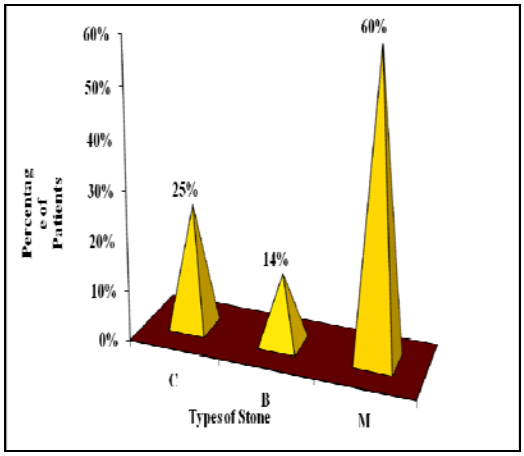

Figure 4. Types and percentage of gallstones. M: Mixed C: Cholesterol B: Bilirubin.

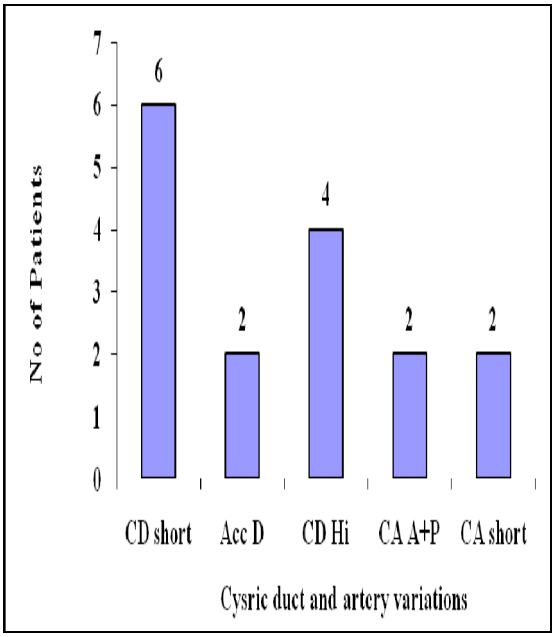

Figure 5. Number of patients with cystic artery and cystic duct variations
CA: Cystic artery.
CD: Cystic duct.
ACC D: Accessory duct.
A+P: Anterior and posterior branches.

Table 1. The final histopathological diagnoses.

Histopathological diagnosis		No. (%)	M No.	F No.
Acute inflammation		15 (5.4)	2	13
Acute on chronic inflammation		49 (17.8)	11	38
Chronic inflammation	Mild	85 (30.9)	16	69
	Moderate & Severe	103 (37.5)	20	83
No significant histopathological finding (Normal)		10 (3.6)	0	10
Malignancies		3 (1.0)	2	1

Table 2. The associated histopathological findings

Histopathological findings	No. (%)	M No.	F No.
Mucosal ulceration	92 (33.4)	17	75
Dysplasia	5 (1.8)	1	4
Metaplasia	17 (6.0)	5	12
Cholesterolosis	26 (9.4)	6	20
Hyperplasia	34 (12.0)	7	27
Rokitansky-Aschoff sinuses	60 (21.8)	11	49
Oesinophilic infilteration	4 (1.4)	0	4

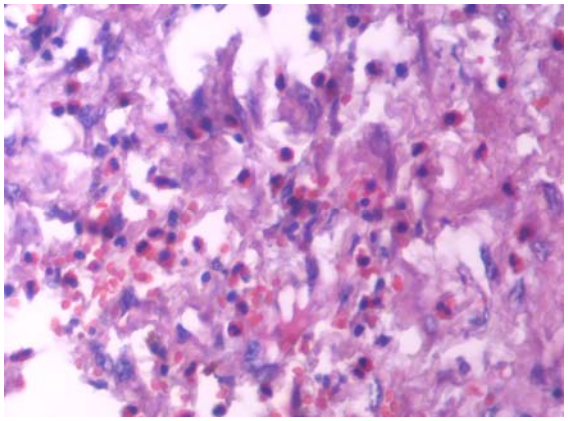

Figure 6. Chronic Eosinophilic Cholecystitis

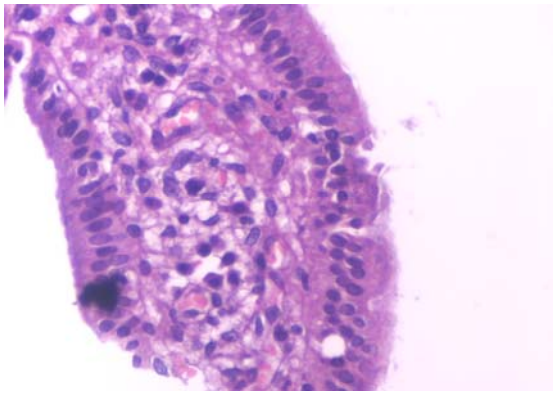

Figure 7. Chronic cholecystitis, there is large number of plasma cells

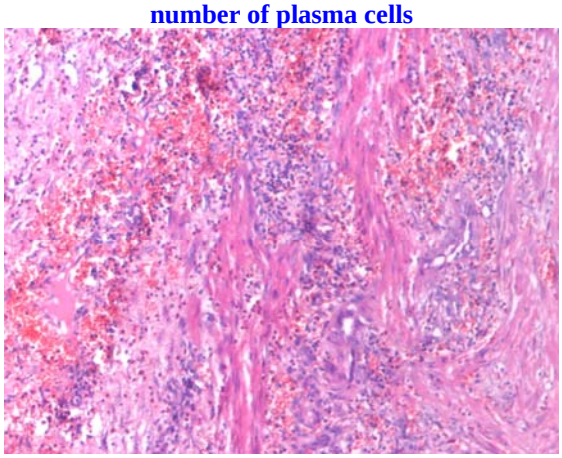

Figure 8. Acute hemorrhagic cholecystitis

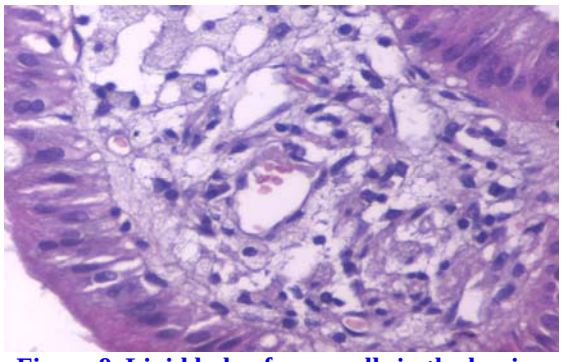

Figure 9. Lipid laden foamy cells in the lamina propria

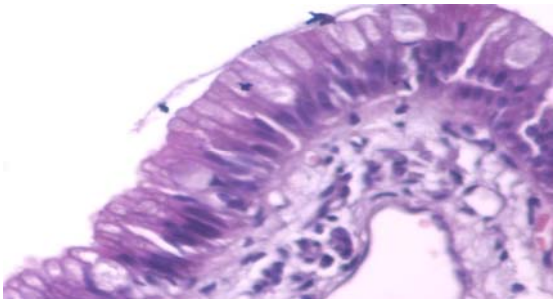

Figure 10. Intestinal metaplasia, many goblet cells are seen in the epithelium

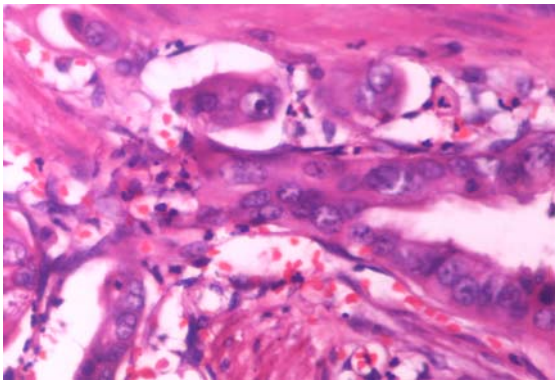

Figure 11. Adenocarcinoma of the gallbladder

DISCUSSION

Gallstones are very common. [3] Gallstone disease is one of the most common problems affecting the digestive tract. [1]

This study finds out the commonest age group underwent cholecystectomy for cholelithiasis is (30-39) years (25.9%).

In this study, patients under twenty years age group constitute only 1.8%, two of them have haemolytic diseases. It is well known that patients with haemolytic anaemia show an increased prevalence of pigment stones. [4]

Haemolytic disease is no longer the predominant risk factor for symptomatic gallstone disease in children. [6]

The most commonly involved age group for cholelithiasis (51%) 41-60 years with females being more common than males. [10]

In the present study, it is found that gallstone disease more common in females then males in a ratio of 4.3:1. Probably this

is related to fertility and the hormonal factors. This is comparable with Al-Khayatt who studied 1000 patients and found a female to male ratio is 3.5:1. [11] It is also comparable to study done by Mustafa and Qibi [12] and Mahmoud and AL-Sakkal. [13] Al-Qazzaz found female to male ratio of 5:1. [14] Zahrani and Mansoor studied the incidence of gallbladder diseases in Saudi Arabia and stated that female to male ratio is 4.6:1. [1]

Volzke et al reported that female gender, age, being overweight is major risk factors for gallstone formation. [16]

Ramzis et al postulated that in USA the prevalence of gallbladder diseases in white women is about 2:1[17] .The predominance of gallbladder diseases among females is reported by Brunicardi [1], Morris [4], Russel [18], Bateson [19] and Kissane [20].

Anatomical variations and congenital anomalies were encountered in [16] patients in the present study.

It was found in relation to cystic artery and cystic duct variations; cystic artery of short type and cystic artery of anterior and posterior branches were seen in (2 patients), short cystic duct in (6 patients), accessory cystic duct (cholecysto-hepatic duct) in (2 patients) and high insertion cystic duct in (4 patients).

Recent studies by Larobina and Nottle concluded that anatomical variations were uncommon and anomalous ducts were not seen. Vascular variations were found to be the only significant abnormalities [21].

Standring reported the variation in the origin of the cystic artery and insertion or drainage of cystic duct, double or absence of cystic duct and one or more accessory hepatic ducts which are rare or uncommon, but on the other hand, these variations in the cystic duct are of considerable importance during surgery [22].

Gallstone chemical analysis revealed mixed type (60%), cholesterol (25 %) and pigment (14%). Multiple gallstones constitute (75%) and the rest was of single type.

Selvi et al found the most common type of stone was the mixed type (55%) followed by pigment and cholesterol stones [10].

Cholelithiasis produces diverse histopathological changes in gallbladder mucosa namely acute inflammation, chronic inflammation, glandular hyperplasia, granulomatous inflammation, cholesterolosis, dysplasia, and carcinoma [23].

In this study, histopathological results were acute inflammation (5.4%), acute on chronic inflammation (17.8%), mild chronic inflammation (31%), moderate and severe chronic inflammation (37.5%), malignancy (1%) and normal histology in (3.6%).

Chronic cholecystitis is the most common finding in the present study (68.55%) followed by acute on chronic, acute, normal and malignancy consequently. This is comparable with a study by Rubin and Farber who stated that chronic cholecystitis is the most common gallbladder disease [24].

Selvi et al reported that chronic cholecystitis was the most common histopathological diagnosis [10].

Strasberg reported about two thirds of patients with gallstone disease present with chronic cholecystitis. Acute cholecystitis is secondary to gallstone in (90-95%) of cases.[25]

The pathologic changes in chronic cholecystitis which often do not correlate well with symptoms, vary from an apparently normal gallbladder with minor chronic inflammation in the mucosa, to a shrunken, non functioning gallbladder with gross transmural fibrosis and adhesions to nearby structures. The mucosa is initially normal or hypertrophied, but later becomes atrophied, with the epithelium protruding into the muscle coat, leading to the formation of the so-called Rokitansky-Aschoff sinuses. [1]

In the present study, associated findings were observed during histopathological examination; and these include mucosal ulceration, cholesterolosis, dysplasia,

metaplasia, hyperplasia, oesinophilic infiltration and Rokitansky-Aschoff sinuses. The later seems to be the most common finding which seen in 60 patients (21.8%), the least common finding is oesinophilic infiltration (4) patients (1.5%).

It is observed in this study that Rokitansky-Aschoff sinuses and glandular hyperplasia were mostly associated with mixed or cholesterol stones. This is compatible with study done by Baig et al who reported this association and explained by that cholesterol may be a more potent stimulus for glandular hyperplasia or the later may responsible for formation of cholesterol rich stones. [23]

In this study, only three patients are recorded with carcinoma of the gallbladders which already have gallstones, this constitutes (1.8%).

Grobmyer et al, Pandey and Shukla stated that cancer of the gallbladder is a rare malignancy that occurs predominantly in the elderly. It is an aggressive tumor, with poor prognosis. It is (2) to (3) times more common in females than males and peak incidence is in the seventh decade of life. However, no statistically significant comment can be made about the sex distribution of biliary carcinoma in this study because of the limited number of patients affected. Approximately (1%) of patients undergoing cholecystectomy for gallstone diseases are found incidentally to have gallbladder carcinoma. [26,27]

Serra et al postulated that cholelithiasis is the most important risk factor for gallbladder carcinoma and up to (95%) of patients with carcinoma of the gallbladder have gallstones. [28]

The conclusions which are made from this study are; gallstones are more common in women; female to male ratio is (4.3:1), the peak age is (30-39) years, chronic cholecystitis is the commonest clinical and histopathological finding which constitutes (68.3%) of the included cases, malignancies of the gallbladder are not common in this study (1%) and all of them were associated with gallstones and anatomical anomalies of the biliary system deserve special attention because of their surgical importance.

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پوختە

بەرکێن زەرافی: ڤەکولینەکا تە خته ڤانی و پوشەکی یە ل سەر 275 نەخوشا ل باژێرێ دهوکێ

باگراوندێ ڤەکولینێ: نەخوشیا بەرکێن زەرافی ژ نەخوشیێن بەربەلاڤێن سیستەمی حەلکرنێ. راپورتێن شیکرنا ئەناتومی، بەربەلاڤبوونا بەرکێن زەرافی ب ڕێژا (11٪ – 35٪) دیار دکەت. وئەڤ بەربەلاڤبوونە گرێدای چەند ئاکامەکا یە ژوانا رەگەز و تەمەن و بنەماکێت مروڤی.بەرکێن زەرافی ژمارەکا گهورینێن پوشەکی کرن ل سەر پوشەکێ نیسەکێ کیسکێ زەرافی ژوانا هەودانا دژوار و کەڤنار یا کیسکێن زەرافی، زێدەبوونا گرێیا، وهەودانا گوگوکا، و نەخوشیا ڤەمانا کلوستروڵی، و تێکدانا پوشەکا و پەنجەشێرێ.

ئارمانجێن ڤەکولینێ: ئارمانجێن ڤێ ڤەکولینێ ڤاڤارتنا رەگەزی و تەمەنی یا نەخوشیا بەرکێن زەرافی ل باژێرێ دهوکێ و دیارکرنا جوداهی بێن ئەناتومی د جوکا زەرافی و گرنکترین جوداهی بێن پوشەکێ کیسکێ زەرافی د دەمی هەبوونا بەرکێن زەرافی تێدا.

رێکێن ڤەکولینێ: ڤێ ڤەکولینێ 275 نەخوش بخوڤە گرت بون، ژوانا 51 د نێر بون و 224 د مێ بوون. و کریارا ژێڤەکرنا کیسکێ زەرافی بو هەمیا هاتبو کرن. و ئەو بەر هاتبونە شیکرن ژلایێ کیمیایی ڤە، هەروەسا نمونێن کیسکێن زەرافی هاتنە وەرگرتن بو شیکرنا پوشەکی.

ئەنجام: دڤێ ڤەکولینێ دا دیار بو کو توشبوونا بێن مێ پتر بو ژ بێن نێر ب ڕێژا (1:4.3) و پتریا ڕێژا توشبوونێ د ژیێ (30–39) سالیێ بوون. هەروەسا دیار بو خو بەرکێن تێکهەل پتریا هەمیان بوون برێژا 60.3٪ و د دویفدا بەرکێن کلوستروڵی برێژا 25٪. جوداهی بێن ئەناتومی د جوکا زەرافی و گەهێن وی برێژا 4.3٪ و 1.4٪ د دویف ئێك دا. هەروەسا ڤێ ڤەکولینێ دیارکر کو پتریا ئەنجامێن شیکرنا پوشەکی ژ هەودانا کیسکێ زەرافی یا بەردەوامە ب ڕێژا (68.3٪) وکێمترین رێژە بێن پەنجەشێرێ بون (1٪).

گەنگەشە: ئەنجامێن ڤێ ڤەکولینێ دیاربون کو وەکی پتریا ڤەکولینێن بەرێ یە و نە وەکی کێمەکا یە. سەبارەت بێن وەکی ئێك کو دگرێدای رەگەزی وتەمەنی بێن توشبووبێن بەرکێن زەرافی هەروەسا کارتێکرنا وان ل کیسکێ زەرافی. وسەبار جورێ بەرکی کا د تێکهەلن یان کلوستروله یان بویاغا زەرافی یە ، دیار بو کو جوداهی بێن هەین دکەل ڤەکولینێن دی.

دەرئەنجام: ژڤێ ڤەکولینێ دهێتە دیارکرن کو بەرکێن زەرافی د رەگەزی مێ دا پترن ژ یێ نێر هەروەسا د تەمەنێ 30–39 سالێ پتریا هەمیا بو. هەروەسا پتریا ڤان نەخوشیا ژ هەودانا کیسکێ زەرافی یێ بەردەوامە و یا کێمترین توشبون یا پەنجەشێرێ یە. جوداهیێن ئەناتومی گرنگیەکا مەزن یا هەی د نشتەرگەریێ دا.

الخلاصة

حصى المرارة: دراسة سريرية نسيجية على 275 مريضا" في مدينة دهوك

خلفية البحث: يعد مرض حصاة المرارة من المشاكل الشائعة التي تصيب الجهاز الهضمي. فقد بينت تقارير الفحص التشريحي شيوع حصاة المرارة بنسبة (11٪) الى (35٪) وأن شيوعها يتعلق بعدة عوامل منها الجنس والعمر والاصول الاثنية. تسبب حصاة المرارة عددا" من التغيرات النسيجية المرضية في النسيج المخاطي لكيس الصفراء ومنها الالتهاب الحاد والمزمن في كيس الصفراء ،التكاثر الغددي ،الالتهاب الحبيبي ،داء تكدس الكوليسترول، خلل التنسج والاورام السرطانية.

أهداف البحث: ان الهدف من هذه الدراسة بيان التوزيع الجنسي والعمري لمرض حصاة المرارة في مدينة دهوك واظهار الاختلافات التشريحية في القناة الصفراوية اهم التغيرات النسيجية في كيس الصفراء في حال وجود الحصى فيها.

طرق البحث: تضمنت هذه الدراسة بيان التوزيع 275 مريضا" منهم 51 ذكرا" و224 انثى وقد اجريت لهم جميعا" عملية استئصال كيس الصفراء لوجود الحصى فيها. وقد اخذت الحصى للتحليل الكيمياوي واخذت عينات كيس الصفراء لاجراء الفحص النسيجي.

النتائج : اظهرت هذه الدراسة أصابة الاناث أكثر من الذكور بنسبة (1:4.3) وأن أكثر الاعمار أصابة هو العقد الرابع أي (30–39) سنة. ولقد أستنتج أن الحصاة الخليطة هي أكثرالانواع شيوعا"بنسبة (60.3٪) تليها حصاة الكوليسترول (25٪).وأن الاختلافات التشريحية في القناة الصفراوية وشرايينها تتضمن اختلافات قناة كيس الصفراء وشريان كيس الصفراء بنسبة (4.3٪) و (1.4٪) بالتعاقب. كذلك اظهرت الدراسة أن اكثر نتائج الفحوص النسيجية المرضية هو التهاب كيس الصفراء المزمن حيث يشكل (68.3٪) واقلها الاورام السرطانية التي شكلت نسبة (1٪).

المناقشة: تبين أن نتائج هذا البحث مطابقة لكثير من البحوث ومغايرة لبعضها . ففيما يتعلق بالتوزيع العمري والجنسي لحصى المرارة وكذلك فيما يتعلق بآثارها على كيس الصفراء ولكن فيما يتعلق بنوع الحصى من حيث كونها خليطة او كوليسترول او صبغية صفراوية فهناك بعض الفرق في هذه الدراسة وغيرها.

الاستنتاجات: يمكن أن يستنتج من هذه الدراسة ان حصى المرارة هي أكثر شيوعا" في النساء من الرجال وفي العقد الرابع من العمر اكثر من غيره. وأن اكثر الحالات المرضية هي التهاب كيس الصفراء المزمن واقلها هي الامراض السرطانية التي تعتبر نادرة وتعتبر الاختلافات التشريحية على اهمية جراحية كبيرة .

INCIDENCE OF *CAMPYLOBACTER JEJU*NI INFECTIONS IN DUHOK CITY

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ABSTRACT

Background and objectives: The incidence and antibiotic resistance of *Campylobacter jejuni* Infections continues to rise worldwide. The aim of this study was to detect the incidence of *Campylobacter jejuni* Infections in infant's diarrheic specimens, poultry cloacal swabs, and environmental surface raw water from Duhok regions. Also, determination of susceptibility test and biotyping patterns of the isolates was studied.
Methods: A total number of 572 samples were collected, from June to November 2006, consisted of 267 samples from infant's diarrheic specimens, 177 samples from poultry cloacal swabs, and 128 samples from environmental surface raw water from Duhok regions. Isolation and identification of the organisms was carried out by conventional standard methods.
Results: The results confirmed 48 (8.39%) isolates as *Campylobacter jejuni* out of 572 collected samples. Of them, 30 (62.5%) isolates were from poultry cloacal swabs, 9 (18.75%) isolates from infant feces, and 9 (18.75%) isolates from surface raw water samples. Biotyping scheme showed predominate biotype I comprising 64.6%, followed by biotype II, III and IV with percentages 27.1%, 6.2% and 2.1% respectively. Susceptibility rates were variable as ciprofloxacin 91.6%, followed by gentamicin 70.8%, chloramphenicol 66.7%, nitrofurantoin 75%, erythromycin 45.8%, co-trimoxazole 43.3%, ampicillin 16.6% and cephalothin 2.1%.
Conclusions: Low incidence rate of *Campylobacter jejuni* in infants feces and surface raw water samples while high proportion were in poultry cloacal swabs .most Isolates showed diversity in biotyping patterns with highly sensitivity to ciprofloxacin and highly resistant to ampicillin and cephalothin.

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Key words: *Campylobacter jejuni*, Incidence, Biotyping, Antibiotics.

Campylobacter jejuni is gram-negative, microaerophilic bacteria colonizing intestinal tract of different animals and poultry birds. [1] The majority of *C. jejuni* infections are sporadic; however, the most common human infections are acute bacterial gastro-enteritis, especially in neonates, infants, young children, elderly age and immunocompromised patients. Although they are usually self-limiting in healthy adults, but complications resulting from Campylobacteriosis also occurred such as reactive arthritis, pancreatitis, and Guillain-Barre′ and Miller-Fisher syndrome in approximately 0.1% of all cases.2

Surveys have suggested that in developed regions, the incidence of infection continue to rise, and exceed the number of reported cases of Salmonellosis. However, the major source of human infection is the handling and consumption of contaminated poultry meat, although epidemiological data suggest other sources of infection like raw milk and untreated surface water implicated as sources or vehicles. [3]

Most human infections with *C. jejuni* are self-limiting and do not require antimicrobial chemotherapy. However, treatment with antibiotics is needed in individuals with invasive or very severe disease.[4,5] Resistance may arise during treatment of humans, but it is also believed that the use of these antibiotics in the veterinary field contributes to increased resistance among strains infecting humans. [6]

The aims of this study were first to detect

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the incidence of *C. jejuni* infections in infant's diarrheic specimens, poultry cloacal swabs, and environmental surface raw water from Dohuk regions. The second aim was to determine antibiotic sensitivity tests and biotyping patterns of the isolates.

METHODS

Samples Collection: From June to November 2006, a total of 572 samples were investigated which distributed between 267 samples from infant's diarrheic clinical specimens, 177 samples from poultry cloacal swabs, and 128 samples from environmental surface raw water from Duhok region. Infant's diarrheic specimens were obtained from those infants suffering from watery diarrhea and/or mucus with or without bloody diarrhea which were admitted to Heevy Children Hospital in Duhok city. The samples were collected using clean containers with screw-cap containing Cary-Blair broth, and dispensed in 3ml aliquots. Poultry cloacal swabs were obtained from Duhok regions (Kalakchi, Qasrok and Semeel town) using sterile swabs inserting into broiler cloacae and fresh cloacae droppings, break off the handle of swabs and placed in bottles containing Cary-Blair transport broth, tighten caps, and dispensed in 3ml aliquots. Environmental surface water samples were collected from ducts, streams, ponds (particularly those near to chicken house) in Duhok regions (Kalakchi, Qasrok town) using sterile container and poured directly into bottles contain Cary-Blair broth and dispensed in 3ml aliquots. All the collected samples were transported in cold environment using transport plastic bag contained ice blocks, to Microbiology laboratory in the College of Medicine, Duhok University within the same day, for sample processing.

Sample Processing: The collected samples were inoculated in selective enriched broth (Hunt broth) containing 5% defibrinated sheep blood, Campylobacter selective supplement antibiotic (Butzler's), and Campylobacter growth supplement (FBP), dispensed in 3ml aliquots in bottles with loosen caps. Incubation lasted for 18 hrs at 42˚C in microaerobic condition (5% O2, 10 % Co2, and 85% N2) using Gas-pack generating kit (Campy-Gen-Oxoid) in anaerobic jar. One-two loopful of the enrichment broth was streaked onto two selective agar media: The first medium was Brucella agar with 5% sheep blood, Campylobacter-growth supplement (FBP) and Campylobacter selective supplement antibiotic (Butzler's). The second medium was charcoal cefoperazon agar. Both media were incubated in microaerobic condition in anaerobic jar at 42˚C for 2-3 days. [7]

Identification of *Campylobacter jejuni*:

The growth was identified after 48-72 hrs of incubation, and suspected colonies were identified as a Campylobacter by examining for characteristic morphology and motility using microscope. The presumptive Campylobacters were further identified by traditional methodology and biochemical tests. Three-five suspected colonies were enriched in Brucella broth, enhanced with FBP supplement, in tube with loosen caps incubated in microaerobic condition for 18hrs then streaked on Columbia agar plates for species-level identification of isolates by traditional methodology including oxidase test, catalase test, nitrate reduction test, H2S production on triple sugar iron agar, susceptibility to nalidixic acid (30µg) and cephalothin (30µg) and hydrolysis of hippurate. [(7)]

Biotyping Scheme of *Campylobacter jejuni* Isolates: In the present study, Lior (1984) provisional scheme was used for biotyping of *C. jejuni* isolates, [(8)] as indicated in (Table 1).

Antibiotic Susceptibility Testing: Screening for antibiotic resistance was investigated using modified Kirby &

Bauer methods as described by Wittwer et al (2005). [9]The following antibiotic-impregnated discs (Oxoid) were used;

Table 1. Biotyping scheme of *C. jejuni* isolates.

Biotypes	Hippurate hydrolysis test	Rapid H2S test	DNA hydrolysis test
I	+	—	—
II	+	—	+
III	+	+	—
IV	+	+	+

ampicillin (25µg), cephalothin (30µg), erythromycin (30µg), gentamicin (10µg), nitrofurantoin (30µg), chloramphenicol (30µg), co-trimoxazole (5µg) and ciprofloxacin (5µg).

RESULTS

This investigation was conducted in Duhok city from June to November 2006. Out of 572 samples collected from three different sources, only 48 isolates were found to be *C. jejuni*, representing 8,39% of the total isolates.

The results of isolation and distribution of *C. jejuni* according to sources showed that *C. jejuni* was successfully isolated from 3.37% infants′ diarrheic specimens, 16.95 poultry cloacal swabs and 7% surface raw water samples as shown in (Table 2). The results of biotyping scheme of *C. jejuni* isolates in the present study revealed that there were four biotypes. Biotype I (HipO+, $H2S^{-}$, and $DNase^{-}$) was dominant (64.6%) over the other three biotypes of *C. jejuni*, while the percentages of biotype II (HipO+, $H2S^{-}$, and DNase+) , biotype III (HipO+, H2S+, and $DNase^{-}$), and biotype IV(HipO+, H2S+, and DNase+) were 27.1%, 6.2%, and 2.1% respectively as shown in (Table 3). All isolates (48) of *C. jejuni* were tested to eight different types of antibiotics discs.

Table 2. The incidence rate of *C. jejuni* from various sources.

Source of Infections	No. of Samples	No. of Isolates (%)	% among all positive isolates
infant′s diarrheic specimens	267	9 (3.37)	18.75
Poultry cloacal swabs	177	30(16.95)	62.5
Surface raw water samples	128	9 (7.03)	18.75
Total	572	48 (27.33)	100

Table 3. Percentage of four biotypes of *C. jejuni* in overall isolates (48).

No. of Isolates	Biotype I (%)	Biotype II (%)	Biotype III (%)	Biotype IV (%)
48	31 (64.6)	13 (27.1)	3 (6.2)	1 (2.1)

Table 4. Antibiotic susceptibility test of *C. jejuni* isolates.

Antibiotics	Code	Sensitive		Resistance	
		No.	%	No.	%
Ciprofloxacin	CIP	44	91.6	4	8.4
Nitrofurantoin	F	36	75	12	25
Gentamicin	CN	34	70.8	14	29.2
Chloramphenicol	C	32	66.7	16	33.3
Erythromycin	E	22	45.8	26	54.2
Co-trimoxazole	SXT	21	43.7	27	56.3
Ampicillin	AM	8	16.6	40	83.4
Cephalothin	KF	0	0.0	48	100

The results of antibiotic resistance screening test as shown in (Table4) showing that most 91.6% isolates of *C. jejuni* were susceptible to ciprofloxacin followed by gentamicin 70.8%, chloramphenicol 66.7%, nitrofurantoin 75%, erythromycin 45.8%, co-trimoxazole 43.3%, ampicillin 16.6% and cephalothin 2.1%.

DISCUSSION

Many epidemiological studies confirm that the incidence rate of this bacterium is more than other species, and varies according to the sources of infection, route or vehicles of transmissions, and susceptible host. [10] For example, in a study conducted in Sulymania city, Iraq, it was found that out of 105 stool samples from diarrheic children subjected to culture only 3% was due to *C. jejuni*. [11] In Mosul city, Iraq, two studies reported that the incidence rate of *C. jejuni* in infants diarrheic stool were 4.3% and 13.8% respectively.[12] Our findings are also consistent with a study done in Mosul. [13] This study already accords with numerous published studies reported by. [14,15,16,17,18,19,20] The present study revealed that the incidence rate of *C. jejuni* was 16.95% in poultry cloacal swabs. Similar results clarified by. [13,21,22] The results of our work revealed that 7.03% of *C. jejuni* was detected among surface raw water samples collected from environmental samples such as ducts, streams, ponds (particularly those near to chicken house). Other study showed analogue results. [(23)] Fastidious microaerophilic nature of this bacterium may encounter low rate detection. [24]

It was found that biotype I was dominant over other three biotypes. Moreover, biotype I (17 isolates) had more frequency in poultry cloacal samples (poultry origin) which may be considered as a source of infection. Our results are comparable with studies carried out by, [13, 25] and with that work done in Brazil and Peru by [26, 27] they found out biotype II was more frequent than biotype I and III.

Most of the isolated *C. jejuni* (91.6%) were susceptible to ciprofloxacin as indicated in our study. Ciprofloxacin acts by binding to one of the several DNA synthesis enzymes such as DNA gyrase, DNA topoisomerase IV. [28] In addition resistance to ciprofloxacin may occur by mutation of the chromosomal genes that code for gyrase. Two studies in Mosul city, Iraq, carried out individually reported that the resistance rates to ciprofloxacin were 11.1% and 25% respectively. [12,13] Studies in Pakistan and India showed diverse results since the percentage of resistant of *C. jejuni* strains to ciprofloxacin were 5.5% and 71.4% respectively. [18,19] Low-rate resistance to ciprofloxacin in the present study may be due to restricted use of this antibiotic in childhood age and also in veterinary field in our locality.

The rate of resistance to β-lactam ring antibiotics in this study was very high since the resistance rate to ampicillin and cephalothin was (83.4%) and (97.9%) respectively. In Sulymanya city, the resistance to ampicillin and cephalothin in *C. jejuni* strains of human origin was (28.6%) and (100%) respectively. [11] The results of this study were similar to those observed by Al-Tahi and Hassan. [12,13] Another study reported much higher frequency (85%) of ampicillin-resistant C. jejuni strains of poultry origin. [30] A study in Australia has shown the resistant rate of *C. jejuni* strains from broilers farm to ampicillin was (19.2%).[31] Resistance is usually associated with β-lactamase production, although other mechanisms of resistance could be involved, such as modified penicillin-binding proteins or impermeability. [32]

Resistance rate to gentamicin was low in this study 29.2% which is much higher than study In Sulymanya city, Iraq (0%).[11] But near that of in India (33.3%), [29] and Slovenia (26.4%). [30]

In general, this study concluded that the relative wide range of antibiotic resistance in the present study might be due to misuse, abuse of antibiotics by our patients and the frequently prescribed antibiotics in our clinical practices. It is also believed that the prudent use of these antibiotics in the veterinary medicine for treatment and prevention of infections and in animal feeds as growth promoters has further engendered the development of resistant pathogens which may contribute to multiple-antibiotic resistance among strains infecting humans. [30] As a result, antimicrobial resistance can prolong illnesses and compromise treatment of patients with bacteremia and severe complications. [33]

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پوختە

بە لافبوونا بە کتریای *Campylobacter jejuni* ل پارێزگەها دهوکێ

پێشەکی و ئارمانج ئە م توێژینەوە یە: جیاکردنەوە ودەستنیشانکردنی بەکتریای *Campylobacter jejuni* لە دەرچەی منداڵی توشبوو بە زگچوونی، کۆئەندامی جوتبونی پەلەوەرەو نمونەی وەرگیراو لە ئاوی خاوی سەرزەوی لە خۆ دەگرێت، بە کارهێنانی رێگە ی کلاسیکی.

ریکا بکاریناني: کۆی گشتی نمونە پشکنراوەکان بە رێگەی کلاسیکی 572 نمونە بوون. ئەم نمونانە لە ماوەی نێوان مانگی حوزەیران تا کانونی یەکەمی سالی 2006 کوگرانەوە. نمونەکان، دابەش بووبوون بە سەر68 نمونەی وەرگیراو لە دەرچەی منداڵی توشبوو بە زگچوونی لە نەخۆشخانەی هیڤی منداڵان لە شاری دهوك، 177 نمونەی وەرگیراو لە کۆئەندامی جوتبونی پەلەوەرە و128 نمونەی وەرگیراو لە ئاوی خاوی سەرزەوی لە پارێزگای دهوك. نمونەکان هە لگیران و لە هەمان رۆژدا گواستراندنەوە بۆ تاقیگەهە وپشکینان بۆ کراو دەستنیشانکران.

ئەنجام: رێگەی دەستنیشانکردنی کلاسیکی بریتی بوو لە ئەنجامدانی پشکنینی شێوەیی وچاندن لە سەر ناوەندی جیاکاری و تایبەتمەند و پشکنینی زیندە کیمیایی، کە بە هۆیانەوە 48 جیاکاراوەی بەکتریای *C jejuni* دەستنیشانکران لە کۆی 572 نمونەی وەرگیراو، وەبە رێژەی 8.39٪ کە تیایدا 16.94٪ (30) جیاکاراوە لە کۆئەندامی جوتبونی پەلەوەرە، 3.37٪(9) جیاکاراوە لە دە رچەی منداڵی توشبوو بە زگچوونی ، وە 7.03٪(9) جیاکاراوە لە ئاوی خاوی سە رزەوی بوون. زیندە چاپکردن (biotyping) بۆ 48 جیاکاراوەی بەکتریای *C jejuni* ئەنجامدرا بە بەکارهێنانی سیستەمی Lior سالێ 1984 . ئەنجامەکان دەریانخست کە زیندە چاپی یەکەم (I) زیندە چاپێکی زالە و31 جیاکاراوە لە خۆ دە گرێت بە رێژەی 64.6٪، بە دوایی ئەودا، زیندە چاپی دووەم (II) دێت کە 13 جیاکاراوە دەگرێتە خۆی و بە رێژەی 27.1٪. بە دوایی ئەمانیشدا زیندەچاپی(III) سێ یەم وچوارەم (IV) دێت بە رێژەی 6.2٪ و2.1٪ یەك لە دوای یەك. پشکنینی هەستیاری دژە زیندەگی بەکتریای*C jejuni* بۆ (8) دژە زیندەگی تاقیکرایە وە و ئەنجامەکان دەریانخست کەوا سەرجەم جیاکراوەکان هەستیاریەکی بەرزیان هەبوو بۆ دژە زیندەگی Ciprofloxacin, هەروەها بە رگریەکی بەرزیان هەبوو بۆ دژە زیندەگی یەکانی Ampicillin & Cephalothin، لە کاتێکدا ئەنجامی جۆراوجۆر هەبوو بۆ دژە زیندەگی یەکانی تری وەك Erythromycin, Co-trimoxazoal ,Gentamicin, Chloramphenicol, Nitrofurantoin. ئەم ئەنجامانە جەختیان لە سەر کەم بەکارهێنانی Ampicillin& Cephalothin کردەوە لە چارەسەرکردنی هەوکردنی گەدەیی توشبو بە هۆی ئەم بەکتریایە لە منداڵاندا.

دەرئامانج: لەم توێژینە وەیە، پەیوەندی نێوان چوار زیندە چاپە کەلەسەرچاوەی جیاواز وەرگیرابوون و هەستیاران بۆ دژە زیندەگی یەکان توێژرایەوە. زیندە چاپی یەکەم زیندە چاپێکی زالە و هەستیاریەکی بەرزیان هەیە بۆ Ciprofloxacin, Gentamicin Nitrofurantoin, Chloramphenicol هەروەها بەرگریەکی بەرزیان هەیە بۆ Cephalothin, Co-trimoxazoal, Ampicillin.

الخلاصة

نسبة حدوث أصابات بكتريا *Campylobacter jejuni* في محافظة دهوك

الخلفية والهدف: تناولت الدراسة الحالية عزل وتشخيص الجراثيم *Campylobacter jejuni* من خروج الأطفال المصابين بالاسهال الدموي أو المائي, ومسحات من مجمع الدجاج, وعينات من المياه السطحية الخامة باستخدام الطريقة التقليدية.

طريقة العمل: بلغ مجموع العينات المدروسة باستخدام الطريقة التقليدية 572 عينة جمعت خلال فترة مابين شهر حزيران وحتى شهر كانون الأول2006. هذه العينات توزعت على 67 عينة من خروج الأطفال المصابين بالاسهال الدموي أو المائي في مستشفى هيفي للأطفال في دهوك, 177 عينة من مسحات مجمع الدجاج, اضافة الى 128 عينة من المياه السطحية الخامة في محافظة دهوك.هذه العينات حال جمعها تم نقلها الى المختبر. وقد اعتمدت الطريقة التقليدية على اجراء اختبارات شكلية وزراعية وكيموحيوية .

النتائج :تم الحصول على 48 عزلة شخصت على انها جراثيم *C jejuni* من مجموع عزل 572 وبنسبة عزل (8.39%) منها (30)(٪16.94) من مجمع الدجاج cloacal swab, (٪3.37) (9) عزلات من خروج الأطفال المصابين بالاسهال المائي أو الدموي و (٪7.03) (9) عزلات من نماذج المياه السطحية الخامة.اجريت التنميط الحيوي (biotyping) لعزلات (48) للجرثومة C jejuni مستخدمين نظام Lior 1984 ومعتمدين على: تحلل الهيبورايت, تحلل الحامض النووي DNA, وانتاج H2Sالسريع. وأظهرت نتائج التنميط أن النمط الحيوي الأول هو النمط السائد ضمن مجموع (48) عزلة بلغ عدد العزلات الواقعة ضمن هذا النمط الحيوي (31) عزلة وبنسبة (٪64.6), يليها النمط الحيوي الثاني (13) عزلة وبنسبة ٪27.1 ثم النمطين الحيويين الثالث والرابع وبنسب (٪6.2), (٪2.1) على التوالي. وأثناء دراسة حساسية جرثومة *C jejuni* تجاه بعض المضادات الحيوية (8 مضاد حيوي) أبدت جميع العزلات حساسية عالية تجاه المضاد الحيوي ciprofloxacinوكذلك مقاومة عالية تجاه المضادين الحيويين Ampicillin& Cephalothin في حين كانت النتائج متباينة للأنواع الاخرى من المضادات الحيوية منها gentamicin, chloramphenicol, nitrofurantion, erythromycin, Co-trimoxazoal . حيث أن نتائج هذه الاختبار تسلط الضوء على الاستخدام المحدود للمضاد الحيوي Ampicillin, Cephalothin في علاج حالات الالتهابات المعوية الناجمة من جرثومة *C jejuni* لدى الأطفال.

الاستنتاجات: نسبة تواجد بكتريا *C jejuni* في عينات الاسهال المائي لدى الاطفال وكذلك عينات المياه السطحية كانت قليلة مقارنة بعينات من مجمع الدجاج. أظهرت جميع العزلات انماط حيوية متباينة كذلك ابدت حساسية عالية تجاه المضاد الحيوي Ciprofloxacinبينما لوحظت مقاومة عالية تجاه كلا من Ampicillin& Cephalothin.