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CONTENTS

IMMUNOHISTOCHEMISTRY; AN IMPORTANT DIAGNOSTIC AID IN THE EVALUATION OF OVARIAN CANCER

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ABSTRACT

Background Ovarian cancer is a heterogeneous disease representing variable tumors of different biological behavior, the origin of most of them is poorly understood. This fact makes the definite diagnosis of some ovarian tumors, including secondary tumors, a difficult challenging task.
Aim: Apply immunohistochemistry (IHC) in the diagnosis of different ovarian cancer types, especially those with similar histopathologic (H.P) morphology or poor differentiation.
Methods: This study included 61cases of ovarian cancer diagnosed and reviewed histopathologically in the central lab of Duhok/ Iraq, from July 2008 to November 2011. Different immunohistochemical markers were used for different cases to identify the type and the origin of the tumor. The selection of these combinations based mainly on preliminary histopathological findings .
Results: With the aid of IHC the provisional diagnosis of ovarian cancer was changed in 18 cases (29.5%). Sixteen of them proved to be metastatic cancers from different parts of the body. The colon possessed the commonest site of origin (14.8%). In two cases the IHC changed the diagnosis from one primary type to another.
Conclusion : Histopathologically, ovarian cancer is among the most complex of all human malignancies. The IHC technique affirmed to be an important diagnostic aid to differentiate the primary and secondary ovarian cancer and to verify the specific type of the tumor in the poorly differentiated malignancies.

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Key words: Immunohistochemistry, Ovary, Cancer, Diagnosis

Ovarian cancer is the second most common gynaecologic cancer, and the most lethal type among them in the Western world.[1] The aggressiveness and the late diagnosis in most types are responsible for an overall 5-year survival of less than 30%.[2] Morphologically and biologically ovarian cancer is a heterogeneous disease, which has likely contributed to difficulties in defining the origin and molecular alterations associated with its development and progression. [3]On the basis of morphological criteria, ovarian cancer is classified into five categories; surface epithelial carcinoma, sex cord-stromal tumors, germ cell, mixed (contain elements of more than one tumor), and metastatic one. This classification dictates many aspects of management, and prognosis. [4] The large group of surface epithelial carcinoma includes major types like serous carcinoma (comprises about one-half of all ovarian cancers), mucinous, endometrioid, and others. The sex cord-stromal tumors include estrogens-producing granulosa cell and Sertoli-Leydig cell tumor, accounting for 8% of ovarian cancer. Although the germ cell tumors represent approximately 30% of ovarian tumors, they account for only 5% of ovarian cancer, because most germ cell tumors are teratomas and most teratomas are benign.

Occasionally the definite diagnosis may be difficult or impossible histopathologically, as the tumors may show overlapping features. Since the IHC is an excellent technique to detect exactly the location of proteins in the examined tissue, it could be of great value to assess the type and origin of malignant cells. [5] In specific situations secondary tumors represent diagnostic

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challenge area. The examples not only include poorly differentiated tumors but also the moderately differentiated or even so well differentiated types that can be mistaken for primary tomur with similar morphology. [6]

METHODS

This study was conducted from the first of July 2008 to the first of November 2011. The study included 61females diagnosed as patients having ovarian cancer, 52 of them diagnosed by histopathological examination of ovarian specimens referred to the central lab of Dohuk/ Iraq, in addition to 9 cases referred to the same center as slides and paraffin embedded blokes (PEBs) for second opinion and reevaluation. Pathological examination and classification carried out in a routine fashion using the revised World Health Organization histologic classification for ovarian neoplasms. [7]

Representative PEBs contain the ovarian cancer tissues were selected to perform the immunohistochemical staining protocol according to the Avidin Biotin Complex (ABC) detection system. [8]

Sections of 4 microns thickness, obtained from the PEBs, placed on positively charged slides together with adjacent parallel control sections which were processed with each set of staining for the IHC. Primary and secondary antibody kits were used, provided by the DAKO Company detected with the Envision+ system that employs peroxidase-labeled polymer conjugated to anti-mouse immunoglobulin antibodies. Immune complexes were identified by using a peroxidase reaction with DAB+ as chromogen (Envision+ detection system, K4006, Dako Corp, Carpinteria, CA). The markers were used in panels of different combinations according to the different preliminary histopathological results using the few distinguishing microscopic characteristics. In some cases a second panel was used according to the IHC results of the first panel. The following markers were used in the IHC analysis: cancer antigen 125 (CA-125), cytokeratin 7 (CK7), cytokeratin 20 (CK20), carbohydrate antigen 19-9 (CA19.9), Wilms tumor protein 1 (WT-1), carcinoembryonic antigen (CEA), vimentin , cluster of differentiation 99 (CD99), pan-keratin (PK), cluster of differentiation 45 (CD45), inhibin, alpha-fetoprotein (AFP), gross cystic disease fluid protein-15 (GCDFP-15), estrogens receptor (ER), complexin 2 (CPX2), human melanoma black 45 (HMB45), Anti-Placental alkaline phosphatase (PLAP), Beta human chorionic gonadotropin (beta-hCG), thyroid transcription factor-1 (TTF-1), chromogranin, S100 and actin.

RESULTS

Among the 61 ovarian cancer cases examined histopathologically, 22 cases diagnosed as poorly differentiated carcinoma, 14 mucinous adenocarcinoma, 9 serous adenocarcinoma, 5 yolk sac tumors. The diagnosis favored granulosa cell tumor, stromal tumor and dysgerminoma three cases for each type and undifferentiated malignancy in two cases. After performing IHC staining for all cases, the diagnosis changed in 18 cases (29.5%). In 16 cases (26.2%) the diagnosis changed from primary ovarian cancer into secondary (Table 1). Seven cases of the poorly differentiated carcinoma appear to be metastatic malignancy from different parts of the body; three cases originated from stomach, two from colon, one from fallopian tube and one from the breast. Seven out of the 14 cases (50%) of mucinous adenocarcinoma appear to be metastatic tumors. Only one case of the serous adenocarcinoma verified to be originated from fallopian tube. One of the two undifferentiated malignancies proved to be secondary involvement of the ovary by lymphoma.

Table 1. Preliminary histopathologic (H.P) results versus tissue of origin by IHC results. Cross-tabulation.

	IHC results		
Preliminary H.P results	**Primary**	**Secondary**	**Total**
Poorly differentiated carcinoma	**15 SEC***	**7**	**22**
	68.2%	**31.8%**	**100.0%**
Mucinous adenocarcinoma	**7**	**7**	**14**
	50.0%	**50.0%**	**100.0%**
Serous adenocarcinoma	**8**	**1**	**9**
	88.9%	**11.1%**	**100.0%**
Yolk sac tumor	**5**	**0**	**5**
	100.0%	**.0%**	**100.0%**
Granulosa cell tumor	**3**	**0**	**3**
	100.0%	**.0%**	**100.0%**
Stromal tumor	**3****	**0**	**3**
	100.0%	**.0%**	**100.0%**
Dysgerminoma	**3**	**0**	**3**
	100.0%	**.0%**	**100.0%**
Undifferentiated malignancy	**1*****	**1**	**2**
	50.0%	**50.0%**	**100.0%**
Total	**45**	**16**	**61**
	73.8%	**26.2%**	**100.0%**

** Changed to Surface Epithelial Carcinoma which include the mucinous and serous adenocarcinoma*
*** One of the three cases changed from sex cord-stromal tumor to primary mixed ovarian tumor*
****Proved by IHC to be Granulosa cell tumor in addition to the 3 cases diagnosed by H.P examination*

The colon was the commonest primary site for metastatic cases (Table 2). Regarding the age at time of diagnosis, there was no significance difference between the mean ages of patients with primary and secondary malignancy (Table 3). The IHC changed the diagnosis in other 2 cases from one primary type to another. One of the 2 undifferentiated malignancy affirmed to be granulosa cell tumor which increased the diagnosed of granulosa cell tumor to 4 cases. In one of the three cases of sex cord-stromal tumor the IHC analysis convert the diagnoses to primary mixed ovarian tumor.

DISCUSSION

Ovarian carcinoma is one of the most complex malignancies histopathologically and immunochemically.[8] This fact has been attributed to the heterogeneous origin of the cells; moreover, unlike cells of most other organs the ovarian cancer cells contain a complex malignant differentiation progression. [10]

Histological features facilitate the distinction between different types of primary and secondary tumors, but in some cases it might be difficult or impossible to decide the diagnosis with certainty by histopathology alone. [11]

In our study depending on the IHC analysis, secondary carcinoma was affirmed in 16 out of the 61 cases (26.2%). Our results show that the colon conquered the highest site for secondary metastasis (representing the primary in 56% of secondary tumors and 14.8 of all malignancies).

Table2. Preliminary H.P result versus definite IHC diagnosis Cross-tabulation.

Preliminary H.P results	IHC definite diagnoses						
	Secondary colon	Secondary gastric	Secondary tubal	Secondary breast	Granulosa cell tumor	Mixed tumor	Lymphoma
Poorly differentiated carcinoma	2	3	1	1	0	0	0
	9.1%	13.6%	4.5%	4.5%	.0%	.0%	.0%
Mucinous adenocarcinoma	7	0	0	0	0	0	0
	50.0%	0%	0%	0%	0%	0%	0%
Serous adenocarcinoma	0	0	1	0	0	0	0
	0%	0%	11.1%	0%	0%	0%	0%
Stromal tumor	0	0	0	0	0	1	0
	0%	0%	0%	0%	0%	33.3%	.0%
Undifferentiated malignancy	0	0	0	0	1	0	1
	0%	0%	0%	0%	50.0%	0%	50.0%
Total	9	3	2	1	1	1	1
	14.8%	4.9%	3.3%	1.6%	6.6%	1.6%	1.6%

Table 3. The age in respect to the primary & secondary ovarian tumors.

	Tissue of origin	Number	Mean	Std. Deviation	Std. Error Mean
Age	Primary	45	42.8444	18.37607	2.73934
	Secondary	16	42.0625	13.46338	3.36584

Colon and ovarian carcinomas can be difficult to distinguish histologically from each other in ovarian masses, in peritoneal carcinomatosis, and in metastases to distant lymph nodes. [12,13]

Misdiagnosis in this context may result in delayed identification of the primary lesion or misdirected clinical procedures. Incorrect diagnosis may also lead to inappropriate therapy because metastatic colon cancer is generally treated differently from the primary ovarian cancer. [14]

Antila R et and Singh N reported in their studies that approximately 10–30% of ovarian tumors are metastatic, of these, colorectal metastasis accounts for approximately 4%.[15]Their results were in agreement with our findings.

In congruent to our findings, Seidman JD et, reported that metastatic mucinous carcinomas are more common than primary ovarian mucinous carcinomas in routine practice. [16]

A lower figure of secondary ovarian cancer (7%) has been reported with a higher incidence of Krukenberg i.e. gastric primary origin (a common mistake is to name all peritoneal metastases from any gastrointestinal cancer as Krukenberg cancer) but this is only the case if it originates from primary gastric cancer. [17]

In our study, the role of IHC in detecting the secondary colonic cancer metastasized to the ovary was magnificent. McCluggage WG and Wilkinson N concluded in their study that the use of different markers was an adjunctive diagnostic tool to differentiate secondary colonic cancer from primary ovarian mucinous adenocarcinoma, in addition to clinical history and gross and microscopic findings. [18]

Primary ovarian adenocarcinomas, including those of mucinous types, are usually CK7 positive and CK20 negative, whereas colorectal adenocarcinomas are generally CK7 negative and CK20 positive. [12]

However, occasionally colorectal adenocarcinomas, particularly the poorly differentiated and right-sided tumors, can be CK20 negative. [19]

Furthermore, adenocarcinomas of the appendix, small intestine, and stomach can be CK7 positive. Thus, immune-stains for CK7 and CK20 should be interpreted with caution, always in the light of all clinical information, and with the understanding that no tumor shows absolute consistency in its staining. Therefore, a CK7-positive/CK20-negative immune-profile favors a primary ovarian carcinoma, whereas a CK7-negative/CK20-positive suggests metastatic adenocarcinoma. [20]

It is stressed that the value of CK might be increased when used as part of a larger panel, which may include antibodies to CEA, CA125, and CA19.9. The use of combinations of different kinds of Immunohistochemistry markers was mandatory to differentiate between different types of the ovarian tumors. That was the reason behind using different markers to the recruited cases to approach the accurate diagnosis between primary and secondary ovarian tumors. [21]

Baker PM in his study concluded that most ovarian carcinomas, including those of mucinous type, can be discriminated with high probability from a colorectal carcinoma using a panel of different antibodies directed against CK7 and CK20, as well as Cdx-2, and β-catenin. [22]

Features favoring a metastatic mucinous carcinoma include bilateral involvement, a multi-nodular growth pattern microscopically, ovarian surface involvement (surface implants) and extensive intra-abdominal spread of tumor. However all these features can be seen with primary mucinous tumors and therefore are not reliable for diagnosis. [6]

IHC have changed totally the diagnosis of the 2 cases of undifferentiated malignancies one to secondary lymphoma and the other one to granulosa cell tumor.

Sex cord stromal tumors account for approximately 4% of benign ovarian neoplasms and 7% of primary ovarian malignancies. They have a varied hist-logical appearance and can mimic a wide range of other ovarian neoplasms. [23]

IHC staining was able to change the diagnosis in one of the three cases of sex cord stromal tumors to a mixed type. [21,22]

The distinction between a sex cord tumor and carcinoma with sex-cord-like patterns may be greatly aided by the triad of epithelial membrane antigen (EMA), inhibin, and calretinin, the latter two being typically positive and EMA negative in sex cord tumors, the converse being typical of endometrioid carcinoma Sertoli-Leydig cell tumours and granulosa cell tumours can closely resemble ovarian endometrioid adenocarcinomas, and the juvenile variant of granulosa cell tumour might be difficult to distinguish from small round cell carcinoma including lymphoma. . [18, 21]

CONCLUSIONS

The IHC is of great help in the differentiation between different malignant patterns of ovarian cancer and proved to be a useful ancillary method in the diagnosis and confirmation of primary and secondary tumors, particularly the mucinous type; a challenging aspect of ovarian tumor interpretation. Furthermore, it's of great value in determination of the tissue of origin of the metastatic tumor.

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پوختە

بۆیاغا کیمیایی یا شانەیی یا بەرگریێ IHC، شرۆڤەکرنەکا کلینیکی یا هاریکار و گرنگ بۆ دەستنیشانکرنا پەنجەشێرا هێلکەدانا ئافرەتێ

بەکگراوەند: پەنجەشێرا هێلکەدانێ ل دەڤ ئافرەتێ نەخۆشیەکا فرەنیشانە کو هژمارەکا وەرەمێن خودان ڕەوشتەکێ بایولۆژیێ گوهارتی هەیە, لێ هەتا نوکە ژێدەرێ پتریا ڤان وەرەمان هێشتا نەیێ ڕۆهن و ئاشکەرایە. ئەڤ ڕاستیە دبیتە ئەگەری دەستنیشانکرنەکا هویر بۆ هندەک جورێن وەرەما هێلکەدانێ, هەروەکی وەرەمێن ناڤنجی ببیتە هەڤرکی و کارەکێ ب زەحمەت.

ئارمانج: ب کارئینانا بۆیاغا IHC بۆ دەستنیشانکرنا هژمارەکا جوداجودا یا پەنجەشێرا هێلکەدانێ, ب تایبەت وان جورێن وەکهەڤیەک هەی د تایبەتمەندیێن هێستوپاتولوژی یان وان وەرەمێن ب زەحمەت دهێنە نیاسین د ڕێکێن هێستوپاتولوژیدا.

ڕێك: ڤێ ڤەکولینێ 61 حالەتێن پەنجەشێرا هێلکەدانێ ب خوڤە گرتینە, ئەڤ نەخۆشیە ب ڕێکا هێستوپاتولوژی هاتینە دەستنیشانکرن و دووبارە لێزڤرین ل سەر هاتیەکرن ل تاقیگەها ناڤەندی ل پارێزگەها دهوکێ|هەرێما کوردستانێ, هەر ژ هەیڤا یولیو سالا 2008 تا نوڤێمبەرا 2011. دڤێ ڤەکولینێ دا جورێن جوداجودا یێن بۆیاغا IHC ژبۆ حالەتێن جوداجودا یێن وەرەمێ هاتینە ب کارئینان ژ پێخەمەت دیارکرنا جور و بنیاتێ وێ وەرەمێ. ئەڤ کومەلە هاتینە هەلبژارتن لسەر بنیاتێ گهورینێن شانەیی یێن دەستپێکێ د تێستێن هێستوپاتولوژیدا.

ئەنجام : ل دویڤ ئەنجامێن بۆیاغا IHC ئەنجامێن دەستنیشانکرنا دەستپێکێ بۆ پەنجەشێرا هێلکەدانێ ژ 18 حالەتان (29.5٪), ژ ڤان حالەتان 16 حالەت هاتنە دووپاتکرن وەك حالەتێن ناڤنجی یێن پەنجەشێرێ یێن کو ژ جهەکی لەشی دهێنە ڤەگوهاستن بۆ ئێکێ دی. ڕێژەکا هەرە مەزن دهاتە ڤەگوهاستن بۆ قۆلونێ ئەوژی ب ڕێژا (14.8٪). ژ دوو حالەتان ب کارئینانا بۆیاغا IHC هاتە گهورین کو دەستنشانکرن ژ جورەکێ دەستپێکی بۆ ئێکێ دی.

بدەستڤەهاتن: ژلایێ هێستوپاتولوژیڤە, پەنجەشێرا هێلکەدانێ ئێکە ژ وان وەرەمێن پیس یێن گەلەك ئالۆز ل دەڤ مروڤی, هەردیسان گرنگیا ب کارئینانا تەکنیکا IHC هاتە دووپاتکرن وەك دەستنیشانکەرەکێ هاریکار یێ گرنگ بۆ نیاسین و ژێکجوداکرنا پەنجەشێرا هێلکەدانێ یا دەستپێکی و یا ناڤنجی, هەردیسان ژ بۆ پشت ڕاستبوون ژ جورەکێ دیارکری یێ وەرەمێن پیس ئەڤێن ب سانەهی نەهێنە نیاسین و دیارکرن.

الخلاصة

الصبغه الكيميائيه النسجيه المناعية (IHC)" تحليل تشخيصي مساعد مهم في تقييم سرطان المبيض

الخلفية: سرطان المبيض هو مرض متعدد التباين يمثل أورام مختلفة ذات سلوك بيولوجية متغيرة، ما زال أصل معظمها غير واضح ومجهول. هذه الحقيقة تجعل التشخيص الدقيق لبعض أورام المبيض، بما في ذلك الأورام الثانوية، تحديا ومهمة صعبة.

الهدف:استخدام IHC في تشخيص مختلف أنواع سرطان المبيض، وخاصة الذين لديهم تشابه في خصائص الهستوباثولوجي أو الاورام ضعيفه التمايز في الهستوباثولوجي.

الطرق: شملت هذه الدراسة 61 حاله لسرطان المبيض، تم تشخيصها و مراجعتها بطريقه الهستوباثولوجي في المختبر المركزي في دهوك / العراق، من يوليو 2008 إلى نوفمبر 2011. تم استخدام معلمات مختلفة من IHC لحالات مختلفة من الاورام لتحديد نوع وأصل الورم. تم اختيار هذه المجموعات اعتمادا على التغيرات النسيجية الأولية في فحص الهستوباثولوجي.

النتائج: استنادا الى IHC تم تغيير نتائج التشخيص الاولي لسرطان المبيض في 18 حالة (29.5٪). في ستة عشر منهم تم اثباتهم كحالات سرطان ثانوي منتقل من أجزاء مختلفة من الجسم. وقد احتل القولون المكان الأول (14.8٪). في حالتين غييراستخدام IHC التشخيص من نوع ورم ابتدائي إلى آخر.

الاستنتاج: من الناحيه النسيجية المرضيه سرطان المبيض هو بين الأورام الخبيثة الأكثر تعقيدا عند الإنسان. وقد تم تاكيد اهمية تقنية IHC كتشخيص مساعد هام للتمييز بين سرطان المبيض الابتدائي والثانوي وللتحقق من نوع معين من الأورام الخبيثة ضعيفه التمايز.

ASSESSMENT OF PRESCRIPTION WRITING AT PRIMARY HEALTH CARE CENTERS AND OUTPATIENT CLINICS IN PUBLIC AND PRIVATE SECTORS IN ERBIL CITY

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ABSTRACT

Background and objectives Prescription writing is a science and an art, as it conveys the message from the prescriber to the dispenser. The study aimed at screening of the prescriptions written by physicians for the presence of essential elements.
Methods This study was carried out in six primary health care (PHC) centers, three hospitals (Rizgary, Hawler and Rapareen teaching hospitals), and in three private pharmacies in Erbil city. A cross sectional study was carried out from April 1, 2010 through March 30, 2011. Using convenience sampling method, 1124 prescriptions were collected from the selected health facilities and examined. Comparisons were made between the health facilities.
Results Prescriber name was present on 76.0% of the prescriptions and prescriber address was present on 42.4% of the prescriptions. The name of the patient was present on 98.1% of the prescriptions, whereas the patient's age and sex were mentioned in only 41.3% and 38.1% of the prescriptions respectively. The date of the prescription was provided on 96.1% of the prescriptions, the generic drug name was present only in 6.9% and brand name in 54.6% of the prescriptions. The diagnosis was present in 53.1%.
Conclusions It was concluded that the majority of prescriptions were not ideal.

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Key words: Prescription, drug dispensing, Erbil

Prescription writing is not merely putting few drug names on a piece of paper, rather it is science and art which can be attained only after years of experience, hard work and sound knowledge of the basic subject.[1,2]

Prescribing is a clinical skill that almost every physician practices regularly to attain the desired therapeutic goals. [3] Correct prescription writing has a great influence on the fate of medicine therapy and health of patients. [4] Prescribers are human; therefore make mistakes. [5]

Although the prescription format may vary slightly from one country to another, most countries agree on the core elements that should be included in the prescription order [6-9]. These are: prescriber's name and address, telephone number and signature; patient's name and address, age and weight (important at the extremes of age); prescription date; drug name (preferably generic), formulation, strength, dose, frequency of administration, quantity prescribed, and instructions for patient use. [6-9] In addition, the physician is required to stamp the prescription. The stamp usually contains the name, title and degree of the physician. [10]

Proper documentation of prescribing practice allows the identification of acceptable and non-acceptable prescribing habits. It also helps in setting up monitoring systems to ensure good prescribing practice and to maintain them. Health professionals may also utilize this

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information to develop guidelines for safe and cost-effective prescribing.[10] According to the authors' knowledge the subject was not studied before in Erbil city, and no published work is available.

The objectives of the study were to find out the proportion of prescriptions that contains adequate information about the prescriber (name, telephone no, specialty, department of prescriber, and signature), the patient (name, age, sex, and weight), and the drugs (drug names, strength of medications, dose units, quantity of medications, and instructions for patient use); and finally to compare between PHC centers, hospitals, and private sectors regarding proficiency of writing ideal prescription.

METHODS

Study setting: This study was carried out in six PHC centers (Azadi, Kurdistan, Malafandi, Nafie Akrei, Nazdar Bamarni, Manara), three hospitals (Rizgary, Hawler and Rapareen teaching hospitals), and three private pharmacies (Medica, Hala, and Pak) in Erbil city, Iraq. Erbil governorate is the capital of Iraqi-Kurdistan region. Erbil city was divided into six geographical areas according to the municipalities. From each municipality one PHC center was randomly selected (by simple random sampling method); accordingly six PHC centers were selected. A convenience method of sampling was used to choose the private pharmacies. One of them was chosen by intension of the researchers to be far from the private clinics. Samples were taken from PHC centers and private pharmacies during attending the patients to the pharmacy. Rizgary and Hawler are the only teaching general hospitals in Erbil city, and Rapareen is the only teaching pediatric hospital in the city.

Study design: Cross-sectional study.

Period of study: The study extended from April 1, 2010 through March 30, 2011. Data collection was conducted over a period of six months from Aug 1, 2010 through Jan 31, 2011.

The sample: A convenience sample of 1124 prescriptions were collected from the selected health facilities and reviewed for the presence and accuracy of information contained.

Two methods were used for data collection: in the first method, 314 stored prescriptions in Rizgary and Hawler hospitals were reviewed (273 from Rizgary, and 41 from Hawler hospital); whereas in the second method, an exit interview was carried out (after doctor consultation) with 810 patients (369 from the three hospitals, 344 from the PHC centers, and 97 from the private clinics). Regarding the hospitals' sample, 170 interviews done in Rapareen hospital, 113 done in Rizgary hospital, and 86 interviews done in Hawler teaching hospital.

During the interview, patients were asked whether the doctor examined them or not, and whether the doctor gave them information about the prescription or not. Patients have been asked about their age, in order to compare it with the age that is registered on their prescriptions. Then their prescriptions were examined for the completeness of information.

Compliance with the elements of prescription writing was defined as the degree to which the physician had met the obligation of including all the elements of a prescription in the prescription order. The information written within the prescription was judged "partly clear" if one word or the dose unit was not written clearly and "not clear" if none of the 2 observers present during the screening session can read it (The 1st observer was one of the researchers, and the 2nd observer was the pharmacist).

The questionnaire:
A questionnaire designed by the researchers was used to collect the following information: Prescriber's name and degree, telephone number and signature, department of prescriber, specialty; patient's name, age, sex and weight (important at the extremes of age); prescription date; drug name (preferably generic), formulation, strength, dose, frequency of administration, quantity prescribed, and instructions for patient use.

Ethical considerations:
The interview was done in a friendly environment in the pharmacy with assurance of the privacy and confidentiality of the collected data. A permission to carry out the study was obtained from the Directorate of Health (DOH) of Erbil Governorate. The study proposal was approved by Scientific Committee of the College of Medicine Hawler Medical University and a verbal consent (for an interview) was obtained from participants having prescriptions in their hand.

Data analysis:
Data were analyzed using statistical package for social sciences (SPSS version 18). Chi square test of association was used to compare between three proportions (of PHC centers, hospitals, and private sectors).
A "P" value of ≤ 0.05 was considered as statistically significant.

RESULTS

Prescriber name and address were present on 76.0% and 42.4% of the prescriptions respectively. Prescriber telephone number was present only on 3.2% of the prescriptions, both prescriber department and signature were included in 66.6%, and 97.7% of the prescriptions respectively. Prescriber handwriting was clear in 30.1% of the prescriptions, not clear in 5.9% of the prescriptions and partly clear in 64.0% of the prescriptions as shown in (Table 1).
Results showed that the name of the patient was present on 98.1% of the prescriptions, whereas the patient's age and sex were present in only 41.3% and 38.1% of the prescriptions respectively. None of the prescriptions included weight of the patient.
The date of the prescriptions was provided in 96.1% of the prescriptions, the generic drug name was present only in 6.9% and brand name in 54.6% of the prescriptions, while both were present on the same prescription in 35%, and in the rest (3.5%) of the prescriptions the drug names were not readable. Regarding the strength of medications, it was included for all drugs in 8.5% of the prescriptions. The dose units were not mentioned for all drugs in 69% of the prescriptions, the units were mentioned for all drugs in 8% of the prescriptions. Quantity of medications not included for all drugs in 59.7% of the prescriptions. The majority (94.1%) of the prescriptions contained partial instructions. The diagnosis was present in 53.1%, and missing in 46.5% of the prescriptions as shown in (Table 2).
During patient interview age was compatible in 89.2% of the 446 prescriptions. The prescription was based on clinical examination in 84.2%. Instructions were given to the patient in 42.2% of 810 prescriptions as shown in (Table 3).
(Table 4) shows that the prescriber name was present in 69.5% of the PHC centers, and it was present in about 76.6% of the hospitals, while in private sectors 94.8% of the prescriptions contained prescriber name ($P<0.001$). Prescriber address was present in (6.1%, 53.9%, 90.7%) of the PHC centers, hospitals, and private sectors respectively ($P<0.001$). Prescriber telephone number was present only in 37.1% of the prescriptions in private sectors ($P<0.001$). Signatures were present in (99.7%, 96.6%, 97.9 %) of the PHC centers, hospitals, and private sectors

respectively (P=0.008). Diagnosis was present in 95% of the PHC centers, and in 38.2% of the hospitals, while in private sectors present in 9.3%. The hand writing of the prescriber was partly clear in 78.8%, 56.8%, 61.9% of the PHC centers, hospitals, and private sectors respectively (P<0.001).

Patient's name was present in 100% of the PHC centers, 98% of the hospitals and 92.8% of the private sectors (P<0.001), patient's age was present in 77% of the PHC centers, 25.5% and 25.8% of the hospitals and private sectors respectively (P<0.001). The sex of the patient was not present in private sectors, in PHC centers the sex of the patient was present in 74.1%, while in hospitals the sex of the patient was present in 25.3% (P<0.001). Patient's weight was not present in all health facilities. Date of prescription was provided in 100% of the PHC centers and in 97.7% of the hospitals, while in the private sectors the date of prescriptions was provided in 71.1% (P<0.001) as shown in (Table 5).

Generic drug name was written in 8.1% of the PHC centers, and 7.2% of the hospitals, while in private sectors drugs were not prescribed by their generic names. Brand names were written in half of the prescriptions of the PHC centers and hospitals, and in 67% of the private sectors (P<0.001). Strength of medications was included for all drugs in 4.7%, 5.1%, 46.4% of the PHC centers, hospitals, and private sectors respectively (P<0.001). Dose units were written for all drugs in 4.7% of the PHC centers, and hospitals, while in private sectors dose units were written in 43.3% (P<0.001). Quantity of medications were included for all drugs in 24.2% of the PHC centers, and in 16% of the hospitals, while in the private sectors were present in 53.6% (P<0.001).

Table. 1 Distribution of sample by prescriber information present on prescriptions.

Prescriber information		N	No	%
Name	Absent	1124	270	24.0
	Present		854	76.0
Address	Absent	1124	647	57.6
	Present		477	42.4
Telephone number				
	Absent	1124	1088	96.8
	Present		36	3.2
Specialty	GP		18	1.6
	Specialist	1124	148	13.1
	SHO		311	27.7
	Unidentified		647	57.6
Department of prescriber				
	Absent	683*	228	33.4
	Present		455	66.6
Signature	Absent	1124	26	2.3
	Present		1098	97.7
Prescriber's handwriting				
	Not clear	1124	66	5.9
	Clear		339	30.1
	Partly clear		719	64.0

** For hospitals only*

Table 2. Distribution of sample by drug information present on prescriptions.

Element		No.	% n=1124
Date of prescription			
	Not provided	**44**	**3.9**
	Provided	**1080**	**96.1**
Drug names	**Generic**	**77**	**6.9**
	Brand	**614**	**54.6**
	Mixed	**394**	**35.0**
	Not readable	**39**	**3.5**
Strength of medications			
	Included for all drugs	**96**	**8.5**
	Included for some drugs	**275**	**24.5**
	Not included for all drugs	**753**	**67.0**
Dose units			
	Included for all drugs	**90**	**8.0**
	Included for some drugs	**258**	**23.0**
	Not included for all drugs	**776**	**69.0**
Quantity of medications			
	Included for all drugs	**244**	**21.7**
	Included for some drugs	**209**	**18.6**
	Not included for all drugs	**671**	**59.7**
Instruction for patient use			
	Included for all drugs	**28**	**2.5**
	Included for some drugs	**3**	**0.3**
	Partial instructions	**1058**	**94.1**
	Not included for all drugs	**35**	**3.1**
Diagnosis			
	Missing	**523**	**46.5**
	Not clear	**4**	**0.4**
	Present	**597**	**53.1**

Table. 3 Distribution of sample by patient information present on prescriptions (patient interview)

Patient interview		n	No.	%
Compatibility of age written in prescription:				
	Not compatible	**446***	**48**	**10.8**
	Compatible		**398**	**89.2**
Was prescription based on clinical examination:		**810****		
	No		**128**	**15.8**
	Yes		**682**	**84.2**
Were instructions given to the patient verbally:				
	No	**810**	**468**	**57.8**
	Yes		**342**	**42.2**

** Number of the prescriptions age written on it (patient interview).*
*** Number of sample taken by interviewing with patients.*

Most of instructions were partial, 96.5% of the PHC centers, and 94.3% of the hospitals, while in the private sectors 84.5% of the instructions were partial (P=0.001) as shown in (Table 6).

During patient interview the age was compatible in 87.6% of the PHC centers and in 90.6% of the hospitals, while in private sectors the age was compatible in 100% (P=0.175). Patients were examined in 80.5%, 85.1%, 93.8% of the PHC centers, hospitals, and in private sectors respectively (P=0.005). Instructions were given to the patient verbally in 20.3% of the PHC centers, and in 59.6% of the hospitals, while in private sectors instructions were given in 53.6% (P<0.001) as shown in (Table 7).

Table 5. Proficiency of writing ideal prescriptions (patient information) by type of health care facility.

variables	PHC centers n=344 No.	%	Hospitals n=683 No.	%	Private sectors n=97 No.	%	Total n = 1124 No.	%	P value
Patient name									
Absent	0	0	14	2	7	7.2	21	1.9	< 0.001
Present	344	100	669	98	90	92.8	1103	98.1	
Patient age									< 0.001
Absent	79	23	509	74.5	72	74.2	660	58.7	
Present	265	77	174	25.5	25	25.8	464	41.3	
Patient sex									
Absent	89	25.9	510	74.7	97	100	696	61.9	< 0.001
Present	255	74.1	173	25.3	0	0	428	38.1	
Patient weight									NA
Absent	344	100	683	100	97	100	1124	100	
Present	0	0	0	0	0	0	0	0	
Date of prescription									
Not provided	0	0	16	2.3	28	28.9	44	3.9	< 0.001
Provided	344	100	667	97.7	69	71.1	1080	96.1	

NA: Not applicable

Table 6. Proficiency of writing ideal prescriptions (drug information) by type of health care facility.

Variables		PHC centers n=344 No.	%	Hospital n=683 No.	%	Private sector n=97 No.	%	Total n=1124 No.	%	P value
Drug name	**Generic**	28	8.1	49	7.2	0	0	77	6.9	
	Brand	175	50.9	374	54.8	65	67.0	614	54.6	
	Mixed	141	41.0	227	33.2	26	26.8	394	35	< 0.001
	Not readable	0	0	33	4.8	6	6.2	39	3.5	
Strength of medication										
Included for all drugs		16	4.7	35	5.1	45	46.4	96	8.5	
Included for some drugs		67	19.4	179	26.2	29	29.9	275	24.5	< 0.001
Not included for all drugs		261	75.9	469	68.7	23	23.7	753	67.0	

Dose unit									
Included for all drugs	**16**	**4.7**	**32**	**4.7**	**42**	**43.3**	**90**	**8.0**	
Included for some drugs	**60**	**17.4**	**169**	**24.7**	**29**	**29.9**	**258**	**23.0**	**< 0.001**
Not included for all drugs	**268**	**77.9**	**482**	**70.6**	**26**	**26.8**	**776**	**69.0**	
Quantity of medication									
Included for all drugs	**83**	**24.2**	**109**	**16.0**	**52**	**53.6**	**244**	**21.7**	
Included for some drugs	**40**	**11.6**	**154**	**22.5**	**15**	**15.5**	**209**	**18.6**	**< 0.001**
Not included for all drugs	**221**	**64.2**	**420**	**61.5**	**30**	**30.9**	**671**	**59.7**	
Instruction for patient use									
Included for all drugs	**2**	**0.6**	**22**	**3.2**	**4**	**4.1**	**28**	**2.5**	
Included for some drugs	**0**	**0**	**3**	**0.4**	**0**	**0**	**3**	**0.3**	**< 0.001**
Partial instruction	**332**	**96.5**	**664**	**94.3**	**82**	**84.5**	**1058**	**94.1**	
Missing for all drugs	**10**	**2.9**	**14**	**2.1**	**11**	**11.4**	**35**	**3.1**	

Table 7. Proficiency of writing ideal prescriptions (patient interview) by type of health care facility.

Patient interview	PHC centers n=344 No.	%	Hospital n=683 No.	%	Private sector n=97 No.	%	Total n=1124 No.	%	P value
Compatibility with age written in prescription :									
Not compatible	**33**	**12.4**	**15**	**9.4**	**0**	**0**	**48**	**10.8**	**0.175**
Compatible	**232**	**87.6**	**145**	**90.6**	**25**	**100.0**	**402**	**89.3**	
Was prescription based on clinical examination :									
No	**67**	**19.5**	**55**	**14.9**	**6**	**6.2**	**128**	**15.8**	**0.005**
Yes	**277**	**80.5**	**314**	**85.1**	**91**	**93.8**	**682**	**84.2**	
Were instructions given to the patient verbally :									
No	**274**	**79.7**	**149**	**40.4**	**45**	**46.4**	**468**	**57.8**	**<0.001**
Yes	**70**	**20.3**	**220**	**59.6**	**52**	**53.6**	**342**	**42.2**	

DISCUSSION

A drug prescription is often the endpoint of a patient's visit to a medical practitioner. As an instruction from a prescriber to a dispenser, it is considered to be a medico-legal document that should be written legibly, accurately and completely [6,7] . In this study prescriber name and signature were absent in 24% and 2.3% of the prescriptions respectively, disagree with the study done in Asir region, Saudi Arabia [11] in which 16.7% of the prescriptions were deficient in the prescriber name and 18.1% deficient in the prescriber signature. Also disagree with what was found by Balbaid and Al-Dawood [12] who reported that prescriptions from some Ministry of Health hospitals in Jeddah city were deficient in physician's name and signature in 14% and 16.3% of cases, respectively. Meyer [13] from a

hospital and clinic in Texas mentioned that through a survey sent to 71 outside pharmacies requesting information on problems related to prescriptions indicated that 96% of responders (one physician and one pharmacist) believed that failure to print the prescriber name was one of the main problems. Blatt *et al.* [14] have shown that 20-30% of prescriptions from a central hospital in Yaounde, Cameroon, did not include the name and the degree of the prescriber. Result of this study showed that 3.2% of the prescriptions contained telephone number of prescriber this disagrees with the study done in Asir region, Saudi Arabia [11] in which none of the prescription contains prescriber telephone number. Also finding of current study showed that almost two-thirds (64%) of prescriptions suffered from poor handwriting this is agree with the result of the study done in Asir region, Saudi Arabia [11] in which illegible handwriting reported in 64.3% of the prescriptions and it disagrees to what was found by Balbaid and AL-Dawood [12] in Saudi Arabia who reported illegible handwriting in only 7.2% of the prescriptions. The high percentage of poor handwriting could be due to the fact that the presence even of a single unclear word or a dose unit was considered as poor handwriting for the whole prescription. Poor handwriting is a serious problem that might lead to dispensing error to the patient with serious or even fatal results. [15] Meyer [13] found that 15% of prescriptions studied had illegible handwriting.

Furthermore, in a survey sent to 71 outside pharmacies from a hospital and clinic in Texas, 69% of responders stated that illegible handwriting was one of the main problems they encountered. Makonnen *et al.* [16] of a tertiary care pharmacy in Addis Ababa, Ethiopia also reported illegible prescriptions in 15% of cases.

Concerning patient information, in the current study the prescriptions were deficient in patient's name, age and sex in 1.9%, 58.7% and 61.9% of prescriptions, respectively, this disagrees with the study done in Asir region, Saudi Arabia [11] in which 5.4%, 22.7%, and 48.7% of the prescription were deficient in patient's name, age, and sex, respectively. This might be attributed to that the receptionist, being in a hurry, writes only patient's name, rather his age and sex. Also disagrees with the results of Balbaid and AL-Dawood [12] in Saudi Arabia, their corresponding figures were 14.5%, 10%, and 4.1% respectively; and disagree with what was reported by Bawazir. [17] in a large study from 22 major hospitals from all health regions within Saudi Arabia, patient age was missing in 18.6% of prescriptions, while patient's name and sex were missing in 0.2% of prescriptions. Current study was somewhat similar to what was reported by Makonnen *et al.* [16] about the quality of prescriptions at a tertiary care pharmacy in Addis Ababa, Ethiopia, where 50% of prescriptions did not contain the sex and age of the patient. Francois *et al.* [18] in a French university hospital reported that complete patient information was provided in only 35.5% of prescriptions. In the current study none of the prescriptions reviewed contained weight of patient; inclusion of weight is recommended for

patients at the extremes of age [6-9] because of the implication it has on drug pharmacokinetics and pharmacodynamics.

Finding of this study showed that 3.9% of prescriptions were not dated, this disagree with study done in Asir region, Saudi Arabia [11] in which 64.3% of their prescriptions were not dated. And lower than the results of Balbaid and AL-Dawood [12] in Saudi Arabia and Francois *et al*. [18] in a French university hospital who found that only 8.7% and 4.5% of prescriptions were not dated, respectively. This might be due to the regulations of the DOH that consider presence of date of prescriptions as mandatory for dispensing of medications.

Result showed that doctors rarely (6.9%) wrote the generic names of drugs (8.1% in PHC centers, 7.2% in hospitals, 0% in private sectors). Disagree with the study done in Islamic Republic of Iran in which 98% of GPs used to write generic names of drugs on the prescriptions. [19] However, a number of factors have been attributed to the failure of private doctors to prescribe medicine. Economic factors may play a role, as some pharmaceutical companies pay rewards to doctors who prescribe their products and this discourages generic prescribing. Also in a study conducted in Zimbabwe found that the desire to sustain income, play a role in the prescribing and dispensing habits of private doctors. [20]

The result of this study disagrees with a study conducted in outpatient clinic of a Nigerian public hospital where the average percentage of drugs prescribed by generic names was 49.5%. [21] Using generic names in prescriptions give flexibility to the dispensing pharmacist and may be of economic benefit to the patient. However, use of brand names may be acceptable when problems of drug bio-availability are expected. [6,8] The result of current study was lower than the result observed in health centers in Bahrain in which for only 10.2% of the prescribed drugs was the generic name of the drug used. [22] Also lower than results of a study done in Sudanese hospital in which generic drug names were written in 19.5% of the prescriptions. [23] In this study brand names were written in 54.6% of the prescriptions this results lower than two studies conducted in Hadramout, Yemen, their results were 60.8%, and 68% of prescriptions prescribed by brand names. [24] And agree with the results from a study of health units in Nepal. [25] These results may indicate a strong influence of the pharmaceutical industry on prescribers. [26] The result of this study higher than the study conducted in Asir region, Saudi Arabia in which brand names were recorded in 50.1% of prescriptions. [24]

In this study approximately two-thirds (67%) of prescriptions did not include the strength of medication, the dose units were not included in 69% and the quantity of medications was not included in 59.7% of prescriptions. The result of this study disagrees with Balbaid and AL-Dawood [12] in Saudi Arabia reported that the dose, frequency and duration of medications were deficient in 7.6%, 6.9% and 10.2% of prescriptions, respectively. Also disagrees with Bawazir [17] in Saudi Arabia who reported that the dose of the drug was missing in 4% of prescriptions.

Apparently, these parameters are left to the pharmacist to decide upon and the implications for the duration of therapy will be dependent on the individual pharmacist. The strength of medication is particularly needed when the pharmaceutical product exists in more than one strength. Francois *et al.* [17] in a French university hospital reported that medication information was complete in only 24% of cases, whereas Blatt *et al.* [14] in France recorded that medication information was stated in 85% of outpatient and 50% of emergency room prescriptions.

One of the prescription problems in this study was that doctors paid little attention to the instructions for patient use, which leads to poor compliance, and the majority (94.1%) contained only partial instructions, a finding that certainly will affect the adequacy of therapy. The result of this study was higher than a study conducted in Asir region, Saudi Arabia [24] in which 90.7% of the prescriptions contain partial instructions. And disagrees with Bawazir [17] in Saudi Arabia who reported that instructions for patient use were missing in only 4% of prescriptions.

Result showed that the diagnosis was missing in 46.5% of the prescriptions, this result was higher than the study conducted in Asir region, Saudi Arabia [24] reported that diagnosis was missing in 34% of the prescriptions. Disagree with what was found by Balbaid and AL-Dawood [12] in Saudi Arabia who found that the diagnosis was missing in only 6.8% of prescriptions, and Bawazir [17] in Saudi Arabia who found that the diagnosis was missing in 9.8% of the prescriptions.

The researcher did not come across a study that compare between PHC centers, hospitals, and private sectors. It was found that there were significant differences between PHC centers, hospitals and private sectors regarding presence of information about prescriber name, address, telephone number and specialty. These differences might be due to presence of prescribers own printed forms in the private sector that contain the name, the address, telephone number and specialty of the prescriber, or as a propaganda for the economic benefit of the prescriber, or for patient's follow up reason. There were significant differences in writing the diagnosis and presence of signature between PHC centers, hospitals, and private sectors, both of them written more in PHC centers. This might be due to the regulations of the DOH that consider "diagnosis writing" and "presence of signature" mandatory for drug dispensing. Also there were significant differences regarding patient's information (name, age, and sex) where they were written more in PHC centers. This might again, be due to the regulations of DOH in this respect.

CONCLUSIONS

Results of this study showed that the majority of the prescriptions reviewed were not ideal.

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پوختە

خەملاندنی راچێتەی پزیشکیە (ئەوانەی نووسراون لەلایەن پزیشکەکان) لە بنکەی چاودێری تەندروستی سەرەتاییو کلینیکی دەرەوە ل کەرتەکانی گشتی وتایبەت ل باژێری هەولێری.

پێشەکی و ئارمانج: نووسینی ڕاچێتە زانست و هونەرە کە پەیامی پزیشکە بۆ نەخۆش. هونەری نووسینی راچێتە یەکێکە لە بیروبۆچوونە بنەرەتیەکان کە پێویستە بۆ پزیشك. مەبەست لەم لێکۆڵینەوە یە خەملاندنی راچێتەی پزیشکیە (ئەوانەی نووسراون لەلایەن پزیشکەکان) لە ڕووی پێکهاتە بنەرەتیەکان کەوا پێویستن بۆ راچێتە.

ریکێن ڤەکولینێ: نموونەی لێکۆڵینەوەکە پێکهاتووە لە راچێتە پزیشکیەکان کە وەرگیراون لە (6) بنکەی چاودێری تەندروستی سەرەتایی و لە (3) نەخۆشخانەی فێرکاری (ن.رزگاری.ن.هەولێر.ن.راپەرینی مندلآن) وە هەروەها لە (3) دەرمانخانەی تایبەتی لەناو شاری هەولێر, بە بەکارهێنانی شێوازی پانەبڕگەیی لە سەرەتایی مانگی نیسانی ساڵی (2010) تاکو (30) ئازاری ساڵی (2011) . لێکۆڵینەوەکە لەسەر (1124) راچێتەی پزیشکی ئەنجامدرا لەو دامەزراوە تەندروستیانەی کە پێشتر ئاماژەمان پێ کردن. ئەو زانیاریانەی کەوا لێکۆڵینەوەیان لەسەر کرا بریتی بوون لە: ناوی پزیشکە نووسەرەکە, واژووی, ژمارەی تەلەفۆنی, ناوی نەخۆش, تەمەنی نەخۆش, ڕەگەزی نەخۆش, کێشی نەخۆش, بەرواری نووسینی راچێتەکە, جۆری دەرمانەکە, چڕی دەرمانەکە, پێك هاتەی, شێوازی بەکارهێنانی, بڕی دەرمانەکە, چۆنیەتی بەکارهێنانی لەلایەن نەخۆشەکە.

ئەنجام: ئەنجامەکانی ئەم لێکۆڵینەوەیە پیشانی دا کەوا : 76٪ی راچێتەکان ناوی پزیشکی لەسەر بوو , لە کاتێکدا تەنها 42.4٪ پسپۆری پزیشکەکەی لەسەر بوو. ناوی نەخۆشەکە لەسەر 98.1٪ی راچێتەکان دەبینرا, بەلآم تەمەنی نەخۆش و ڕەگەزەکەی تەنها لەسەر 41.3٪ و38.1٪ بە دوای یەکتر دەبینرا. وەبەرواری نووسینی راچێتەکان لەسەر 96.1٪ی راچێتەکان نووسرابوو, ناوی زانستی دەرمانەکان لەسەر تەنها 6.9٪راچێتەکان نووسرابوو وە ناوی بازرگانی لەسەر 54.6٪ راچێتەکان نووسرابوو. دەست نیشان کردنی (تشخیص) لەسەر 53.1٪ی راچێتەکان ئاماژەی پێ کرابوو.

دەرئەنجام: لێرەوە بۆمان دەردەکەوێت کە نووسینی راچێتەی پزیشکی لە ناو شاری هەولێر ناتەواوە وە پێویستی بە دواداچوون و چاك کردن هەیە .

الخلاصة

تقييم كتابة الوصفات الطبية في مراكز الرعاية الصحية ألأولية والعيادات الخارجية في القطاعين العام والخاص في مدينة أربيل

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.

طرق البحث: تم أخذ نماذج من الوصفات الطبية لغرض دراستها من ستة مراكز للرعاية الصحية الاولية وثلاثة مستشفيات تعليمية (مستشفى رزكارى, مستشفى هولير, مستشفى رابرين للاطفال) وكذلك تم شمول ثلاث صيدليات (ميديكا, هالة, باك) من القطاع الخاص في الدراسة داخل مدينة أربيل خلال فترة الاول من شهر نيسان عام 2010 لغاية 30 أذار, 2011. تم اعتماد 1124 وصفة طبية كعينات لغرض دراستها حيث تم تجميعها من المؤسسات الصحية التي تم ذكرها سابقا وتم تجميعها وتبويب المعلومات الوارد ذكرها في الوصفة, حيث شملت هذه المعلومات: اسم الواصف (الطبيب), شهادته, رقم الهاتف والتوقيع, اسم المريض, العمر, الجنس والوزن وكذلك تاريخ كتابة الوصفة الطبية, نوع الدواء وتركيبه وتركيزه وطريقة الاستعمال والكمية الموصوفة و توصيات لكيفية استخدام الادوية من قبل المريض.

النتائج: أظهرت النتائج ما يلي: تضمنت 76٪ من الوصفات اسم الطبيب الواصف, فى حين ان 42.4٪ من الوصفات تضمنت العنوان الوظيفي للواصف, تم ذكر اسم المريض فى 98.1٪ من الوصفات ولكن عمر المريض وجنسه ذكر فى 41.3٪ و38.1٪ من الوصفات على التوالي. تم ذكر تاريخ كتابة الوصفة فى 96.1٪ من الوصفات, تم استخدام الاسم العلمي للدواء فقط فى 6.9٪ فى حين استخدم اسم التجارى فى 54.6٪ من الوصفات, أما التشخيص فقد ذكر فى 53.1٪ من الوصفات .

الاستنتاجات: نستنتج مما ذكر سابقا ان كتابة الوصفات الطبية داخل مدينة أربيل غير متكاملة وتحتاج الى المتابعة والتحسين.

HUMAN LEUKOCYTE ANTIGEN (HLA) TYPING BY POLYMERASE CHAIN REACTION – SEQUENCE SPECIFIC PRIMERS (PCR – SSP) IN COMPARISON WITH SEROLOGIC METHODS

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ABSTRACT

Objective: This study was conducted to compare the efficacy and accuracy of the molecular Polymerase Chain Reaction – Sequence Specific Primers (PCR – SSP) based method with the serologic methods for HLA typing, in order to choose the best feasible test as a routine tissue typing test in the future in our clinical laboratories concerned to organs transplantation.
Methods: The HLA-I was typed serologically by lymphocytotoxicity method using (HISTO TRAY HLA-I typing Kit, Germany) and the HLA Class I by PCR-SSP method using (HISTO TYPE SSP kit, Germany).
Results: For the serologic method, the frequency of the different polymorphic HLA-I antigens were 44 for locus A, 53 for locus B and 38 for locus C in 20 screened persons (2.2, 2.6 and 1.9 polymorphic HLA antigens per single person respectively), whereas the frequency of the HLA-I (A) locus detected by PCR-SSP was 310 in 70 screened persons which makes 5.2 polymorphic (A) antigens per single person. Also the discrepancy between the two methods was very clear in the serologic method, there was only one (A38 antigen) (2.27%) which was not detected by the PCR-SSP method, while in DNA dependent method (SSP), there were 4 (A) antigen types (A68, A31, A34, and A210).
Conclusions: Amplification of HLA loci with PCR-SSP has proved to be a rapid and accurate method for HLA-A, -B and -C alleles typing. Also PCR-SSP allowed determination of the subtypes of HLA antigens very clearly and in broader scale making the matching of HLA types easier and more precise.

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Key words: Human Leukocyte Antigens (HLA), Tissue Typing, PCR-SSP and lymphocytotoxicity.

The genetic loci involved in the rejection of foreign organs are known as the Major Histocompatibility Complex (MHC). In humans, the MHC is called Human Leukocyte Antigen (HLA) because these antigens were first identified and characterized using alloantibodies against human leukocyte cells [36]. The human major histocompatibility complex (HLA) maps to the short arm of chromosome six (6p21) and spans approximately 3.600 Kilo base of DNA [7]. Based on the structure of the antigens produced and their functions, there are three classes of HLA antigens termed accordingly as HLA Class I, Class II and Class III [31].The cell surface glycopeptides antigens of the HLA-A, -B and -C series are called HLA Class I antigens [30]. The cell surface glycopeptides antigens of the HLA-DP, -DQ and -DR loci are termed HLA Class II [15].The tissue distribution of HLA Class II antigens is confined to the "immune competent" cells, including B-Lymphocytes, macrophages, endothelial cells and activated T-lymphocytes [22,34]**.** The class III region has the highest gene density but some of the genes are not involved in the immune system [3,16]. HLA-A, HLA-B and HLA-DR have long been known as major transplantation antigens. Recent clinical data indicate that HLA-C matching also affects the clinical outcome of heamatopoitic stem cell transplantation, but HLA-DQ and HLA-DP do not appear

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critical [14,28].Since some HLA antigens are recognized on all body tissues (rather than just blood cells), the identification of HLA antigens is described as "Tissue typing". Tissue typing is a procedure in which the tissues of a prospective donor and recipient are tested for compatibility prior to transplantation [8]. There are clear relationships between the degree of HLA matching and organs graft survival in transplants from living related donors [37]. With respect to the extensive variations of HLA molecules and scarcity of mono-specific antibodies for detection of each antigen, HLA-I and HLA-II can be typed at DNA level with more accuracy (fewer errors) and more precise (more discriminating) by molecular techniques compared to serologic typing [4,38]. Variety of Polymerase Chain Reaction based typing such as Restriction Fragment Length Polymorphism (RFLP), Sequence Specific Primers (SSP), Sequence Specific Oligonucleotide Probes (SSOP) and Sequence Based Typing (SBT) have been developed and applied for clinical HLA typing [22].

Amplification of HLA loci with PCR-SSP has proved to be a rapid and accurate method for genotyping HLA-A, B and C alleles [9,12], and indicates that HLA typing by serology may not be sufficiently reliable [2,21]. Serology is a quick and convenient method of HLA Class I detection, but it is hindered in many cases by serological cross reactivity and decreased in expression of HLA antigens, particularly in immunosuppressed patients or in patients with different hematological tumors [11].The aim of this Study is tocompare the efficacy and accuracy of the molecular (PCR-SSP) based method with the serologic routine methods, in order to choose the feasible one as a routine tissue typing test in the future in our clinical laboratories related to organs transplantation units.

METHODS

Sampling : A total of 14 families were selected randomly from Duhok city/ Kurdistan Region/ Northern Iraq, each family constituted of 5 people (the parents and 3 siblings) enrolled in this study. So the total number of the subjects involved was 70 people. Ethical committee approval was obtained prior to the commencement of the study with full consent from people involved in the study. Thirty-five of the total subjects (50%) were males and the other 50% were females. The ages of the siblings ranged between 8-32 years and the parents ranged between 38-75 years. A total of 5ml of peripheral blood was taken from each person involved in the study using disposable sterile syringes, 2.5 ml of blood sample was collected in a heparinized tube from four families (5 person from each) for the serological HLA-I typing as a control for the best histocompatibility match (Group I), whereas the other 2.5 ml was collected from the whole 70 people (Group II) in an EDTA tube and stored frozen for the DNA analysis.

SEROLOGICAL HLA TYPING

The HLA-I was typed serologically by lymphocytotoxicity method using (HISTO TRAY HLA-I typing Kit, Germany). In this test, sample lymphocytes that are previously isolated were added to readily prepared sera (microplates reaction trays that already pre dropped specific anti-HLA antibodies with pre identified HLA-I phenotypes) which may or may not have antibodies directed to HLA antigens. If the serum contains an antibody specific to an HLA class I antigen on the lymphocytes, the antibody will bind to this HLA antigen. Complement is then added. The complement binds only to positive cells i.e., where the antibody is bound, and in doing so, it causes membrane damage [18].

The damaged cells are not completely lysed but suffer sufficient membrane damage to allow uptake of vital stains such as eosin or fluorescent stains such as ethidium bromide. Microscopic identification of the stained cells indicates the presence of a specific HLA antibody. Stained lymphocytes indicate a positive reaction. In case of missing antigen-antibody reaction, the cell membrane remains intact, no penetration of indicator dye takes place and the cells remain unstained indicating a negative reaction. For reading the results, each reaction tray was covered with a cover glass shortly (5 min.) before reading under an inverse contrast microscope [10] .To evaluate the HLA-I type, the amount of lysed lymphocytes (%) in each reaction tray compared with the total amount of lymphocytes was quoted as a score value in each tray as follows:

Table 1. score value.

% Lysed cells		Score	Evaluation
0-19%	=	score 1	negative
20-39%	=	score 2	doubtful negative
40-59%	=	score 3	weak positive
60-79%	=	score 4	positive
80-100%	=	score 5	no evaluation possible

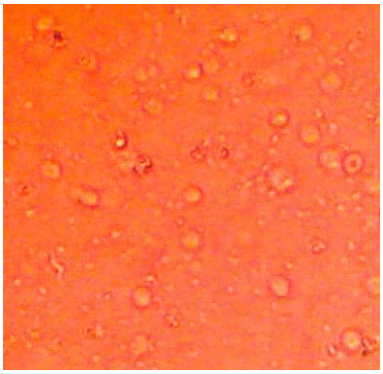
DNA Analysis and Molecular Techniques

DNA was extracted from whole blood samples according to the modified method described by [6]. The concentration of DNA was then determined spectrophotometricaly using UV spectrophotometer. The extracted DNA was used to detect the HLA Class I by PCR-SSP method using (HISTO TYPE SSP kit, Germany). The preparation of DNA master mix and running program in the thermal cycler has performed according to manufacture instructions that are provided with kit. The PCR amplification products were detected using gel electrophoresis (The gel concentration should be 2.0 - 2.5% of Agarose) and detected under UV light source.

RESULTS

According to the scoring Table provided with the kit, all the test wells were examined microscopically and the number of the lysed cells were the indicator of the test evaluation. All the test wells contained 40-100% lysed lymphocytes were evaluated as positive for a defined specific HLA-I phenotype (Figure 1), but the wells contained less than 40% lysed cells were evaluated as negative (Figure 2).

The positive reaction findings were assigned to the specificities of the anti-sera contained in each well that were already defined on provided HLA-I, A, B, and C polymorphism tables (included with each kit), the anti-sera used for the typing are listed in (specific appendix that provided with kit). Each positive reaction occurred was due to the presence of antigens on the lymphocytes corresponded to antibody present in the antisera of HLA class I. There was a clear polymorphism, and most of the samples were typed for HLA-I A, B and C successfully. (Table 2) shows the phenotypes of HLA-I, A, B, and C in all the families tested. The results of HLA-I typing of the whole 20 screened subjects are given in the(Table2), which shows polymorphism in both the loci "A" and 'B" alleles reflecting many sero-specificity, but less in locus "C". In locus "A" there were 44 different polymorphic sero-specificities found, the most frequent one was A10 which represented 15.9% of the total "A" locus typed.


A

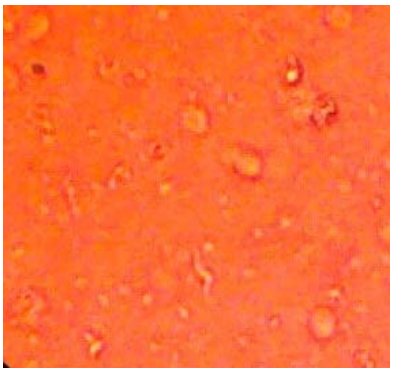
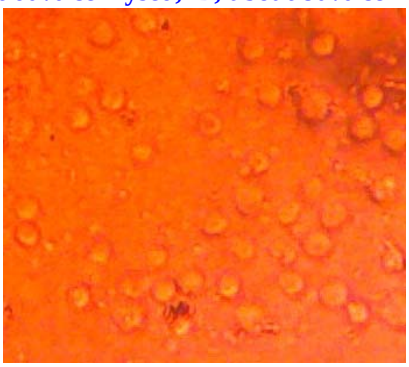

B

Figure 1. Positive results of two different test sample wells for HLA-I specific antigen phenotypes, A; about 60% cell lysed, B; about 80% cell lysed are indicated. (400X).


A

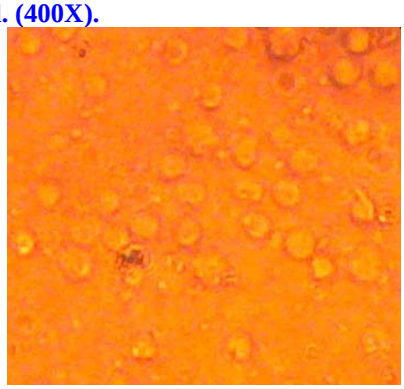

B

Figure 2. Negative results of two different test sample wells for HLA-I specific antigen phenotypes, A; less than 10% cell lysed, B; about 30% cell lysed are indicated. (400X).

Table 2. The HLA-I sero-pattern of each subject in each family. Families No. (5, 6, 7, and 8) are the families correspondent to the same families tested for HLA-I, A by PCR-SSP.

Family No. 5	HLA – A, B, C (loci)		
	A	B	C
Father	A66/A10	B7/B45, B76, B82	Cw1 / Cw6
Mother	A10/ A24 (9)	B8, B59 / B39	Cw5 / Cw6
Sibling 1	A66/ A19	B76 / B39	Cw5/Cw1
Sibling 2	A10/A9	B59 / B7	Cw5 / Cw1
Sibling 3	A24 (9) / A23	B76 / B39	Cw5 / Cw1
Family No.6			
Father	A24 / A32	B5, B51, B52/ B28	Cw5/ Cw7
Mother	A66 / A19	B8, B14, B39 / B33, B31	Cw5/ Cw6
Sibling1	A10 / A30	B8, B59 / B51	Cw3 / Cw5
Sibling2	A9 / A19	B45, B76 / B12 (44)	Cw4 / Cw5
Sibling3	A24 / A19	B51 / B30, B31	Cw6 / Cw7`
Family No.7			
Father	A66 (10) / A43	B8, B59 / B38	Cw2 / Cw7
Mother	A1 / A203	B62 / B52 , B49	Cw1 / Cw2
Sibling 1	A1 / A10	B38 / B52	-----
Sibling 2	A2/ A11	B58 / B52	Cw2 / Cw2
Sibling 3	A1 / A43	B52 / B8	Cw7 / Cw1
Family No.8			
Father	A24 / A3(38)	B52, B49 / B12	Cw2 / Cw4
Mother	A1 / A26	B7 / B8, B59	Cw4 / Cw7
Sibling 1	A26 / A10	B49 / B59	Cw4 / Cw4
Sibling 2	A43 / A3	B52 / B59	Cw4 / Cw2
Sibling 3	A24 / A43	B49 / B12	Cw2 / Cw7

Whereas for the locus 'B", polymorphic alleles reflected greater sero-specificity which was 53 different types, the most frequent one was B59 (13.20%). For the locus "C", usually the polymorphism was much less in comparison with the others. Only the Cw 1, 2, 3, 4, 5, 6 & 7 were typed, and the most frequent one was Cw 5 (21.05%), the sibling 1 in family No.7 were excluded from typing HLA-I,C because most of the results were doubtfully positive as shown in (Table 3).

Table 3. The frequency and percentage of the A, B and C serotypes in HLA class I typing.

HLA class I								
A Locus			B Locus			C Locus		
Serotype	Freq.	%	Serotype	Freq.	%	Serotype	Freq.	%
A10	7	15.90	B59	7	13.20	CW5	8	21.05
A24	6	13.63	B8	6	11.32	CW2	7	18.42
A66	4	9.09	B52	6	11.32	CW1	6	15.78
A9	4	9.09	B76	4	7.54	CW4	6	15.78
A19	4	9.09	B39	4	7.54	CW7	6	15.78
A43	4	9.09	B49	4	7.54	CW6	4	10.52
A1	4	9.09	B51	3	5.66	CW3	1	2.63
A3	2	4.54	B7	2	3.77			
A26	2	4.54	B31	2	3.77			
A203	1	2.27	B45	2	3.77			
A23	1	2.27	B12	2	3.77			
A32	1	2.27	B45	1	1.88			
A30	1	2.27	B82	1	1.88			
A11	1	2.27	B5	1	1.88			
A38	1	2.27	B28	1	1.88			
A2	1	2.27	B14	1	1.88			
			B33	1	1.88			
			B44	1	1.88			
			B30	1	1.88			
			B38	1	1.88			
			B62	1	1.88			
			B58	1	1.88			
Total: 44			Total:53			Total: 38		

Regarding to Molecular Typing (PCR-SSP) of HLA, the specificity table and evaluation diagram included with the kit were used for the evaluation, In all lanes of the electrophoresis gel for each run there was a clearly visible PCR band in 1070 bp size which is reflecting the internal positive control (Figure 3).

Figure (3) represents an example of the gel electrophoresis for family screened for HLA-I, A typing. The upper row of lanes were the PCR results of the father, the next down row lanes represented the PCR results of the mother, and the other three rows represented the PCR results of the three siblings in each family. In all the tests, most of the PCR bands size was measured according to the marker provided from (BAG healthcare).

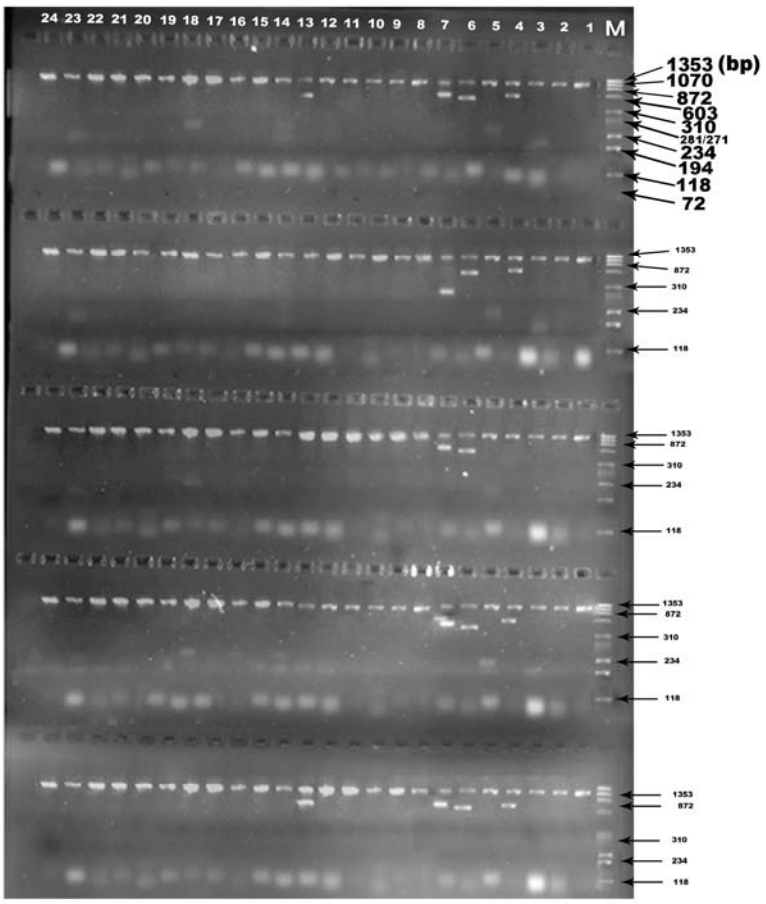


Figure. 3. Represents 2-2.5% agarose gel electrophoresis of PCR-SSP molecular pattern of the subjects typed for HLA-I, A in family number 1 (Lane M; DNA marker; Lanes 1 to 24 are DNA PCR product samples; The band at 1070 bp are the internal positive control).

All of the measured bands were compared and aligned with the evaluation diagram (specific appendix, provided with each kit), and evaluated for the HLA-I, A sero- typing as indicated in (Table 4).

Table 4. Sero-specificity indicated by PCR-SSP DNA band sizes.

Family		HLA-A Locus	
		Allele 1 (sero-specificity according to PCR product band size)	Allele 2 (sero-specificity according to PCR product band size)
	Fath.	A23, (9), Null, A24,	A43
	Moth.	A23 (9), A9, Null	A24 (9), A24, Null
1	S.1	A23 (9), Null, A24 (9), A9, Null	A43
	S.2	A23 (9), Null, A24 (9), A9, Null	A43
	S.3	A23 (9), Null, A24 (9), A9, Null	A66 (10), A (10), A 11
		A2, A203, A210, Null	A29 (19), Null
	Fath.		
2	Moth.	A2, A203, A210, Null	A69 (28)
	S.1	A2, A203, A210, Null	A69 (28)
	S.2	A29 (19)	Not recognized
	S.3	A3, Null	A25 (10), A10 (Doubtful)
	Fath.	A10, A11, Null, A66 (10)	A24 (9),A 24, A9
	Moth.	A3, Null	A80
3	S.1	A3, Null	A10, A11, Null, A66 (10)
	S.2	A24 (9), A24, A9, Null	Doubtful
	S.3	A24 (9) A24, A9	A23, A9, Null doubtful or A11, Null,

			A10, A66, (10)
	Fath.	A24 (9), A(24) , Null, A(9)	---
	Moth.	A11, Null, A10,A 66 (10)	A23 (9), Null, A24 (9), A24 A9
4	S.1	A24 (9), A24, A 9, Null, A 23, A9	A2, A203, A210
	S.2	A24 , Null	A11, Null, A10, A66 (10)
	S.3	A24 (9), Null, A9	A2, A203, A210
		A66(10)	A10
	Fath.		
5	Moth.	A66(10)	A23 (9), A24(9)
	S.1	A66(10)	A31(19)
	S.2	A66(10)	A23(9)
	S.3	A24(9)	A 10, Null (doubtful)
	Fath.	A11, Null,A10,A66(10),A23(9),A24	A32(19)
	Moth.	A11, (Null), A10, A66(10)	A30, A19
6	S.1	A10, A11, A66(10)	A19, A30
	S.2	A10, A11, Null, A66(10)	A23(9), A24(9), A24
	S.3	A23(9), Null, A24(9), A24, Null, A9	A34, A24, A9
		A11 ,Null, A10, A66(10)	A26(10), A10, Null, A43
	Fath.		
7	Moth.	A1, Null	A2, A203, A210, Null
	S.1	A1, Null	A11(Null), A10, A66(10)
	S.2	A2, A203, A210, Null	A11, Null, A10, A66(10)
	S.3	A1,Null	A26(10),43,Null
	Fath.	A24, Null, A9	A3, Null, A68, A(28), Null
	Moth.	A1, Null	A26(10), A10(Null), A43
8	S.1	A26(10), A10, A10(Null)	A25(10), A10, Null, A43, A66(10)
	S.2	A43, A26(10), A10(Null),	A3, Null
	S.3	A24(9), Null	A2, A203, A210, Null
		Not included	
	Fath.		
9	Moth.	Not included	
	S.1	Not included	
	S.2	Not included	
	S.3	Not included	
10		No PCR bands (No Reaction)	
	Fath.	A2, A203, A210, Null	A11, Null, A10, A66(10)
	Moth.	A30(19)	A34(10), A66(10)
11	S.1	A34(10), A66(10)	A74(19), A19, Null
	S.2	A2, A203, A2(10), Null	A30(19)
	S.3	A11,Null,A66(10)	A31(19),Null
	Fath.	A23(9), Null, A24(9), A9, A24	A24(9), A9, A24
	Moth.	A11(Null), A10	A23(9), Null, A24(9), A(9), A24
12	S.1	A11(Null), A10	A23(9), Null, A24(9), A(9), A24
	S.2	A11(Null), A10	A23(9), Null, A24(9), A(9), A24
	S.3	A11(Null)A10	A23(9), Null, A24(9), A(9), A24
	Fath.	A23(9), Null, A24(9), A9	A24(9)
	Moth.	A2, A203, A210, Null	A34(10), A66(10)
13	S.1	A10, A66(10)	A23(9), A9, A24
	S.2	A3, Null	A23(9), A24(9), A9
	S.3	A3, Null	A10, A66(10)
14		No PCR bands (No Reaction)	

According to (Table 4), there were 23 different polymorphic HLA-I A antigens detected and 74 Null antigens. The PCR-SSP pattern of the (father) in family 4, gave a vague result, and so it was excluded from the screening. Also the PCR-SSP pattern of the sibling 3 in family five gave some doubtful results. All of the screened

subjects for HLA-I, A of the family 9 gave the same pattern which could be due to sample contamination. (Table 5) presents the number, frequency and percentage of the different polymorphic HLA-I, A antigens detected by PCR-SSP on all the alleles of the screened subjects.

Table 5. Types, frequency and the percentage of the detected HLA-I, A antigen using PCR-SSP HLA typing method.

HLA-I, A (Locus)	Frequency	%
A9	65	20.96
A10	53	17.09
A24	45	14.51
A66	24	7.74
A23	21	6.77
A11	19	6.12
A2	12	3.88
A203	11	3.54
A210	11	3.54
A19	11	3.54
A43	7	2.25
A3	6	1.93
A1	4	1.29
A30	4	1.29
A34	4	1.29
A28	3	0.96
A29	2	0.64
A69	2	0.64
A25	2	0.64
A80	1	0.32
A31	1	0.32
A74	1	0.32
A68	1	0.32
	Total expressed HLA-I,A antigens : 310	
A, Null (not expressed) antigen	74	19.27

Comparing the results of HLA-I, A typing of the subjects in families 5,6,7 and 8 using both the serologic method and the PCR-SSP method, it was found that the serologic method was able to detect 16 different polymorphic HLA-I,A antigens, and the PCR-SSP method detected 23 different polymorphic HLA-I,A antigens and 26 Null antigens (not expressed) with a higher frequency in the same screened subjects as shown in (Table 6).

Table. 6. Comparison of the A locus between the serologic and PCR-SSP methods in HLA-I typing.

Families	Serologic Method	PCR-SSP Method
Family No.5	A	
Fath.	A66/A10	A66(10) / A10
Moth.	A10/ A24 (9)	A66(10) / A23, A24(9)
S.1	A66/ A19	A66(10) / A31(19)
S.2	A10/A9	A66(10) / A23(9)
S.3	A24 (9) / A23	A24(9) / A Null (Doubtful)
Family No.6		
Fath.	A24 / A32	A11, Null, A10,A66(10), A23(9),A24(9) / A32(19)
Moth.	A66 / A19	A11(Null), A10, A66(10) / A30, A19
S.1	A10 / A30	A10, A11, A66(10) / A19, A30
S.2	A9 / A19	A10, A11,Null, A66(10) / A23(9), A24(9), A24
S.3	A24 / A19	A23(9), Null, 24(9), A24, Null,A9 / A34, A24, A9
Family No.7		
Fath.	A66 (10) / A43	A11,-,Null, A10, A66(10) / A26(10),A10,Null,A43
Moth.	A1 / A203	A1, Null / A2, A203, A210, Null
S.1	A1 / A10	A1, Null /A11, A10, A66(10)
S.2	A2/ A11	A2, A203, A210, Null / A11, Null, A10,A66, A10
S.3	A1 / A43	A1,Null /A26(10), A43,Null
Family No.8		
Fath.	A24 / A3(38)	A24, Null, A9 / A3, Null, A68, A28, Null
Moth.	A1 / A26	A1, Null / A26(10), A10, Null, A43
S.1	A26 / A10	A26(10), A10, A10(Null) / A25(10), A10, Null, A43,A6610)
S.2	A43 / A3	A43, A26(10), A10(Null) / A3, Null
S.3	A24 / A43	A24(9),Null / A2, A203, Null

DISCUSSION

This study is the first one done in Kurdistan Region, Iraq, using serological and PCR-SSP methods for HLA typing. HLA antigens are the major determinants used by the body's immune system for recognition and differentiation of self from non-self (foreign) substances. This system consists of numerous SNPs (Single Nucleotide Polymorphisms) encoding more than 2,000 known alleles [29]. HLA antigens are regarded as the major barriers in transplantation of organ and tissue between individuals. Data obtained by [38] and [24], about the role of HLA matching in renal transplantation has consistently shown a stepwise decrease in graft survival rate with increasing antigen mismatch.

The HLA types of the donors and acceptors have a significant effect on the occurrences of graft versus host disease (GVHD), engraftment failure, and graft-versus-leukemia effect [20]. The actual HLA testing is performed on a blood sample from the patient and potential donors. There are different ways that an HLA typing test can be done.

These methods differ in their ability to detect differences between a patient and donor. HLA typing can be performed by « serologic typing » or by « DNA molecular typing » techniques [35]. In this study, for serologic HLA typing, we used a panel of ready prepared antisera from (BAG-Healthcare, Appendix I); the antisera were pre-dropped in microtitration wells. Twenty subjects belonged to four families (5 each) were screened for HLA-I A, B and C serologically, if any of which is used as a virtual recipient and the others are used as donors, the probability of selecting the best matched donor is twenty multiplied by twenty which makes 400 comparisons.

For DNA analysis by SSP-PCR, a total of 14 families were screened including 5 persons each which makes the total number of the examined subjects 70 persons. Two of the families were excluded from the study because their SSP-PCR product did not show any pattern, which could be due to the lack of DNA in their samples or any defect in the PCR mixture. So, the total number of the subjects included for DNA analysis were 60 persons, the probability of the comparison for any one of them as a virtual recipient with the others as best matched donors was (60 x 60) which makes 1800 matching. The principle of PCR-SSP is that each individual allele (making up a serological specificity) is amplified by a primer pair exactly matched to that region. Specificity is determined by the use of sequence specific primers in which a 3' single-base mismatch inhibits the priming of non-specific reactions. Because Taq polymerase lacks 3' to 5' exonuclease activity, even if primer pairs do anneal non-specifically, they will not amplify efficiently. Thus, only the desired allele or alleles will be amplified [32]. The antisera panel that are used for serologic HLA-I (A, B, C) typing was consisting antisera of 24 different alleles for the locus "A" 39 for the locus "B' and only 7 for the locus 'C" (Appendix I).The reason for HLA-C alleles being undetected by serology is unclear but a possible explanation is the lack of surface expression and a lack of suitable serological reagents [5]. The most likely cause of serologically undefined HLA-C alleles the lack of suitable antisera complete with low cell surface expression. Despite similar messenger RNA levels, HLA-C antigen are expressed on cell surface at approximately 10 % of the level of either HLA-A or B [17].This may be due to in sufficient assembly of HLA-C molecule with 2-Micoglobulin [39].

The antisera were used for the serologic typing of HLA (A, B, C) of 20 persons belonged to four families (each family was composed of the parents and three siblings). In each family it was tried to investigate the best tissue compatibility between the members serologically. For

the molecular typing, the primers used in the low-resolution PCR-SSP method, we used a panel of 24 primers pairs (5' and 3' ends primers) available from (BAG Healthcare, appendix II), they were specific primers for HLA-A locus. The PCR-SSP low resolution method was used to detect the best tissue compatibility between the members of 12 families (5 persons each) including the same families investigated serologically for HLA typing. In the current study, comparison of the results of the serologic method with the those of the PCR-SSP method for the same screened subjects indicated a broader detection of the "A" locus in the HLA class I antigens with a greater polymorphism and frequency with the PCR-SSP method than that found in serologic method, which means a more flexibility and easiness in matching the donors with the recipients. Also it has been found that the PCR-SSP method is more efficient in detecting the non-expressing HLA-I, A genes (the Null antigens), there were 74 Null "A' genes in all the screened persons by SSP method.

Since the introduction of DNA-based human leukocyte antigen (HLA) typing a number of discrepancies with serological typing have been documented. [13] found 42 HLA class I and II Null alleles had been described characterized by a lack of expression of cell surface antigen. These Null alleles can be accounted for by a number of demonstrated molecular mechanisms including insertion, deletion and point mutation and may lead to a nonsense codon, splicing defect or premature stop codon. In a study done by [23], the results of HLA-A, -B and-C typing using serology in 40 normal Iranian individuals were compared to the results of typing with PCRSSP. In serological HLA-typing, they used a panel of antisera from the third Asia-Oceania Histocompatibility Workshop [26]. The large antisera panel used consisted of 23 different alleles for the "A" locus, 49 for the "B" locus and 8 for the "C" locus. In the PCR-SSP low-resolution method, they used 32 different primer pairs for the "A" locus, 27 for the "B" locus and 23 for the "C" locus. In spite of using a very large panel of antisera in the serological method, [23] found that there were at least 16 blank or undefined antigens (9 in the "A' locus and 7 in the "B" locus) and the PCR-SSP low-resolution method allowed the identification of 2 blanks in the "A" locus and 3 blanks in the "B" locus. According to their results, the resolution of HLA-A PCR-SSP method was largely unaffected by cross-reactivity and they were able to obtain correct and exact results in this locus.

In the present study, however, the precision and accuracy of both the serologic method for HLA-I (A, B, C) and the DNA based method for HLA-I,A for the same families were compared only based on the detection of serologically defined HLA-I (A,B,C) alleles serologically , and indeed, the used primers were specific for determination of only (HLA-A) alleles depending on PCR-SSP. Despite of these, there was a difference between the results of the two methods. For the serologic method, the frequency of the different polymorphic HLA-I antigens were 44 for locus "A" 53 for locus "B" and 7 for locus "C" in 20 screened persons (2.2, 2.8 and 1.9 polymorphic HLA antigens per single person respectively), where as the frequency of the HLA-I, A locus detected by PCR-SSP was 309 in 60 screened persons which makes 4.4 polymorphic "A" antigens per single person. But the discrepancy between the two methods was not very clear, in the serologic method, there was only one (A38 antigen) (2.27%) which was not detected by the PCR-SSP method, where as in DNA dependent method (SSP), there were 4 (21.05%) "A" antigen types (A68, A31, A34, and A210) not detected by the serologic method in the 20 screened persons. (These results are highly in favor with the conclusion of [1], when they found a difference between the

results of the two methods of about 43.7%, that indicated the higher error in serology or more accuracy in PCR-SSP for DR typing. Because in molecular typing such as PCR-SSP, the factors including quantity, quality, and viability of the cells, lack of mono specific antiserum, difference in time and temperature of incubation, precision in reading of microplates, and etc, that are variable in serology, do not affect the PCR-SSP [27]. In some other studies a difference rate of about 10- 57% between serology and DNA-based typing methods has been indicated [25,33]. In another study, [19], demonstrated that the difference in results of PCR-SSP and serology was mainly due to an increase in doubtful results by serology, not technical failures or missed antigens. By serology they found that high percentages in incorrect or inclusive assignment were obtained in HLA, DR13, DR14, DR11 and DR12 for HLA class II and A80, A33, A74, and B38 for HLA class I. Their results clearly show that incorrect antigen assignment which account in about (25%) of HLA class I and (40%) of HLA class II serological typing were all resolved by molecular typing.

From viewing of different studies and articles, it is found that the serological techniques and reagents can not detect all currently known alleles, for this reason, the serological typing is often followed by the molecular typing. The molecular typing identifies nucleotide polymorphism, which code for the different allelic variation where as the serological typing shows molecules that are currently expressed on the cell and involved in the immune rejection of the mismatched grafts. However the use of antibodies to detect HLA specificities can support the molecular techniques and solve ambiguities occurring between the expressed and null alleles. Therefore serology may still be an important tool for tissue typing in donor selection [23].

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پوختە

دەستنیشانکرنا ئەنتیجینێن خانەیێن سپی یێن خوینا مروڤی (HLA)ب تەکنیکا زنجیرەیێن ئەنزیمێ پولیمەرەیس (PCR-SSP) ب هەڤبەرکرن دگەل ریکێن سیرولوژی

پێشەکی و ئارمانج: نووسینی ڕاچێتە زانست و هونەرە کە پەیامی پزیشکە بۆ نەخۆش. هونەری نووسینی راچێتە یەکێکە لە بیروبۆچوونە بنەرەتیەکان کە پێویستە بۆ پزیشك. مەبەست لەم لێکۆڵینەوە یە خەملاندنی راچێتەی پزیشکیە (ئەوانەی نووسراون لەلایەن پزیشکەکان) لە ڕووی پێکهاتە بنەرەتیەکان کەوا پێویستن بۆ راچێتە.

ئارمانج : ئەڤ ڤەکولینە هاتیە ئەنجامدان ژ بۆ بەراوردکرنا شیان و هویراتیا کاری یا تەکنیکا کارلیکێن زنجیرەیێن ئەنزیمێ پولیمەرەیس یا گەردی–پرایمەرێن تایبەتێن لدویڤ ئێك (PCR – SSP) بۆ دەستنیشانکرنا ئەنتیجینێن خانەیێن سپی یێن خوینێ یێن مروڤی (HLA) و هەڤبەرکرنا ئەنجامێن ڤێ ڕێکێ دگەل ڕێکێن سیرولوژی. ئەڤ چەندەژی هاتەکرن بۆ هەلبژارتنا باشترین ڕێکا دەستنیشانکرنێ یا گونجایی و شیانێن ب کارئینانا وێ ب سانەهی کو بهێتە ب کارئینان وەك تێستێن ڕۆتینی بۆ دەستنیشانکرنا ئەنتجینان ل تاقیگەهێن پزیشکی یێن دەستنیشانکرا نەخۆشیان کو سەرەدەریێ دگەل تێستێن دی یێن پێدڤی و گرنگ دکەت یێن بۆ چاندنا شانەیان دهێنە ئەنجامدان.

ئامراز و ڕێکا کاری: ئەنتجینێ خانەیێن سپی یێن مرڤی ژ جورێ HLA-I ب ڕێکێن سیرولوژی ب هاریکاریا ڕێکا ژەهراویبوونا خانەیێن لیمفی (لیمفوسایتوتوکسیسیتی) ئەوژی پشتی کو پشتن بەستن هاتیە کرن لسەر (HsitoTray HLA-I Kit, Germany)و ب ڕێکا زنجیرا کارلیکێن ئەنزیمێ پولیمەرەیس کو پشت بەستن هاتەکرن لسەر (Hist Type SSP Kit, Germany).

ئەنجام: ب هاریکاریا ب کارئینانا ڕێکێن سیرولوژی, فریکوێنسێن جودا جودا یێن ئەنتجینێ HLA-I ب ڤی شێوەی بوون : 44 بۆ جهێ A, 53 بۆ جهێ B, 38 بۆ جهێ C, ژ سەرجەمێ وان کەسێن پشکداری وەك خوبەخش دڤێ ڤەکولینێ دا کری کو هژمارا وان 20 کەس بوون.

ب هاریکاریا تەکنیکا PCR-SSP دیاربوو کو فریکوێنسا HLA-I بۆ جهێ A گەهشتە 130 ژ سەرجەمێ وان کەسێن وەك خوبەش بەشداری دڤەکولینێ کری کو هژمارا وان 70 کەس بوون ئەڤ چەندەژی 5,2 جهێن گوهارتی ددەتە مە. جوداهی دناڤبەرا ئەنجامێن هەردوو ڕێکان بۆ دەستنیشانکرنا ئەنتیجینێ HLA-I گەلەك د روهن و ئاشکەرابوون, ل دەمێ ب کارئینانا ڕێکێن سیرولوژی بتنێ ئەنتینەك هەبوو ئەوژی A38 کو ب تەکنیکا PCR-SSP نەهاتە دیارکرن. ل دەمێ ب کارئینانا تەکنیکا PCR-SSP چوار ئەنتیجین ژ جورێ A هاتنە دیتن ئەوژی ئەڤبوون (A68, A31, A34, A210).

ئەنجامێن دوماهیێ: ب ڕێکا ڤێ ڤەکولینێ دیاربوو کو دەستنیشانکرنا ئەنتیجینێن HLA(A,B,C) ب هاریکاریا تەکنیکا PCR-SSP گەلەك بلەز و هویربوون , هەردیسان هاریکاریا دیارکرا هندەك جورێن ناڤنجی یێن ڤان ئەنتیجینان دکەت ب شێوەیەکێ روهن و ئاشکەرا کو ئەم دشێ، پشت بەستنێ بکەینە سەر بۆ دەستنیشانکرنا دگەلئێکگونجینا شانەیی بۆ نشتەگەریێن چاندنا ئەندامێن لەشی.

الخلاصة

تحديد مستضدات الخلايا البيضاء للأنسان (HLA) بواسطة تفاعل البلمرة المتسلسل (PCR–SSP) بالمقارنه مع الطرق المصليه

الأهداف: تم أجراء هذه الدراسه لمقارنة كفائة ودقة طريقة تفعاعلالبلمره المتسلسل (PCR-SSP) الحديثهوالمتبعه لتحديد مستضدات الخلايا البيضاء للأنسان و مقارنتها مع الطرق المصليه لحديد هذه المستضدات وذلك لأختيار أفضل الطرق التشخيصيه الممكن تطبيقها كفحوصات روتينيهلتشخسص المستضد في المختبرات الطبيهالتشخيصيه والتي تتعامل مع الفحوصات الضروريه لزراعة الأعضاء.

ألمواد وطرق العمل: تم تحديد مستضد الخلايا البيضاء للأنسان من نوع –Iبالطريقهالمصليهبأستخدام سمية الخلايا اللمفيه وذلك بالأعتماد على (Hsito Tray HLA-I Kit, Germany) و بطريقة تفاعل البلمرة المتسلسل بالأعتماد على (Hist Type SSP Kit, Germany).

النتائج: باستخدام الطريقة المصلية، كانت تردد HLA-Iالمختلفه كالتالي : 44 للموقع A , 53 للموقع B , و 38 للموقع C, في مجموع الأشخاص الخاضعين للأختبار والبالغين 20 فردا . بأستخدام طريقة PCR-SSP تبين بأن تردد HLA-I للموقع A بلغ 310 في مجموع الأشخاص الخاضعين للفحص والبالغ عددهم 70 فردا مما يعطي 5,2 موقعا متغايرا . كان التمايز واضحا في نتائج الطريقتين المتبعتين في تحديد الHLA-I حيث أنه باستخدام الطريقهالمصليه كان هناك مستضد واحد فقط من نوع A38 والذي لم يتم الكشف عنه بأستخدام طريقةPCR-SSP وعند أستخدام طريقةPCR-SSP تم الكشف عن أربعة مستضدات من نوع A وهي(A68, A31, A34, A210).

الأستنتاج: تبين من خلال هذه الدراسة بأن تحديد مستضدات HLA(A,B,C) باستخدام PCR-SSP تكون دقيقه وسريعه كما وتساعد على الكشف عن الأنواع الثانويه لهذه المستضدات بصوره واضحه والتي يمكن الأعتماد عليها في تحديد التطابق النسيجي لأجراء عمليات زراعة ألأعضاء.

THE RISK FACTORS FOR SIGNIFICANT NON-HEMOLYTIC NEONATAL HYPERBILIRUBINEMIA IN DUHOK

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ABSTRACT

Background: Jaundice is a common neonatal problem. Sixty-five percent of newborns develop clinical jaundice with a bilirubin level above 5 mg/dL during the first week of life. Bilirubin is a potent antioxidant that may help the newborn who is deficient in most antioxidant substances. Hyperbilirubinemia can also be toxic with high levels resulting in an encephalopathy. Identifying infants at risk of developing severe neonatal hyperbilirubinemia and kernicterus is a problem that clinicians have faced since the condition of neonatal jaundice initially was recognized more than 100 years ago.
Aim: To assess the important risk factors for significant neonatal hyperbilirubinemia in Duhok , Iraq Kurdistan Region.
Methods: The study included 160 cases of non–hemolytic neonatal hyperbilirubinemia that needed management with phototherapy or exchange transfusion and 260 controls who were neonates without significant hyperbilirubinemia. Both groups were studied in terms of the maturity, type of feeding, use of oxytocin to induce labor, the presence of cephalhematoma and or bruises and a family history of a sibling who had significant hyperbiliribinemia and results were statistically analyzed using Chi-square test where P less than 0.05 is significant.
Results: Prematurity was present in 71 cases (44%) and 47controls (18%) P <0.001, Birth weight <2500 grams was present in 76 cases (47%) and 78control (30%) P <0.001, Breast feeding was present in 64 cases (40%) and 123 controls (47%) P <0.001, oxytocin was used in 79 cases (49%) and 173 controls (67%) P <0.001,Cephalhematoma was present in 12 cases (8%) and 42 controls (16%) P = 0.015, family history of a sibling with significant hyperbilirubinemia was present in 16 cases (10%) and 10 controls (4%) P= 0.02
Discussion and conclusion: By statistical analysis it was found that prematurity, low birth weight (< 2.5 kg), breast feeding, use of oxytocin to induce labor, the presence of cephalhematoma and/or bruises and a sibling with history of significant hyperbilirubinemia are all significant predisposing factors as agrees with many studies done allover the world.

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Key words: Neonatal Jaundice, non hemolytic Hyperbilirubinemia, prematurity,

Jaundice is a common neonatal problem. Sixty-five percent of newborns develop clinical jaundice with a bilirubin level above 5 mg/dL during the first week of life. Bilirubin is a potent antioxidant that may help the newborn who is deficient in most antioxidant substances. Hyperbilirubinemia can also be toxic with high levels resulting in an encephalopathy (Kernicterus) [1].

Hemolytic disease of the newborn is a common cause of neonatal jaundice. Many newborn infants without evidence of hemolysis become jaundiced. Bilirubin is produced by the catabolism of hemoglobin in the reticuloendothelial system. One gram of hemoglobin produces 35 mg of bilirubin. Sources of bilirubin other than circulating hemoglobin represent 20% of bilirubin production, these sources include inefficient (shunt) hemoglobin production and lyses of precursor cells in bone marrow. Compared with adults, newborns have a twofold to threefold greater rate of bilirubin production (6 to 10 mg/kg/24 hr versus 3 mg/kg/24 hr). This increased production is caused by an increased RBC mass (higher hematocrit) and a shortened erythrocyte life span of 70 to 90 days compared with the 120-day erythrocyte

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life span in adults. Unconjugated bilirubin binds to albumin on specific bilirubin binding sites. The enzyme glucuronyl transferase represents the rate-limiting step of bilirubin conjugation. The concentrations of ligandin and glucuronyltransferase are lower in newborns particularly in premature infants. Bacteria in the neonatal intestine convert bilirubin to urobilinogen and stercobilinogen which are excreted in urine and stool respectively and usually limit bilirubin reabsorption. Delayed passage of meconium which contains bilirubin also may contribute to the enterohepatic recirculation of bilirubin [2].

Identifying infants at risk of developing severe neonatal hyperbilirubinemia and Kernicterus is a problem that clinicians have faced since the condition of neonatal jaundice initially was recognized more than 100 years ago [3] . Bilirubin dissociates from albumin at the hepatocyte and becomes bound to a cytoplasmic liver protein "Y" (ligandin). Hepatic conjugation results in the production of bilirubin diglucuronide, which is water soluble and capable of biliary and renal excretion. [2].

Epidemiologic studies have identified multiple factors that are associated with an increased or decreased risk of severe neonatal hyperbilirubinemia. The risk factors can be related to maternal or infant characteristics and include items from the medical history, labor and delivery record, physical examination, and blood tests. Used in isolation, such factors have limited predictive ability, but combining multiple factors in clinical prediction rules greatly enhances predictive performance. Major risk factors for non hemolytic significant hyperbilirubinemia include: gestational age 35 to 36 wk , a previous sibling received phototherapy, cephalohematoma or significant bruising, exclusive breastfeeding particularly if nursing is not going well and weight loss is excessive and East Asian race. Minor risk factors include: gestational age 37 to 38 wk, jaundice observed before discharge, previous sibling with jaundice , infant of a diabetic mother who has macrosomia, maternal age more than or equal to 25 yr , male sex and prematurity [4].

Aim of the study

To assess the significance of prematurity, low birth weight, breast feeding, oxytocin use, cephalhematoma and bruises, a family history of a sibling with significant hyperbilirubinemia and maternal age as predisposing factors for non-hemolytic neonatal hyperbilirubinemia and to specify those jaundiced neonates who are in need of close follow up and management

METHODS

This case-control study was conducted in Duhok city for the period of one year from 1st of April 2009 to 1st April 2010. Both cases and controls were taken from both Heevi pediatric hospital and neonatal care unit in Azadi hospital.

Two hundred sixty controls were taken as full term and preterm newborn infants in the first two weeks of life without any significant hyperbilirubinemia i.e. no need for phototherapy or exchange transfusion.

One hundred sixty cases were taken as newborn infants within first 2 weeks of life with significant hyperbilirubenemia (needed either phototherapy or exchange transfusion.) but without any evidence of hemolysis neither on ABO and Rh blood grouping nor laboratory studies (i.e. no reticulocytosis or other evidence of hemolysis like decreasing hematocrit).

All the cases and controls were studied in terms of gestational age (according to last menstrual period and ultrasound study), birth weight (1.5-2.5 kg , 2.5-3.99 kg), type of feeding (breast, bottle, mixed), use of Oxytocin during delivery ,the presence of cephalhematoma or bruises (that could be clinically diagnosed , family history of significant hyperbilirubinemia in siblings and the

maternal age (less than 25 yrs or more than25 yrs).

All the cases and controls underwent the following laboratory studies: TSB and differential, blood grouping and Rh of the mother and babies (any ABO or Rh incompatibility case was excluded), CBC and Direct Coomb's test. The results were studied and statistically analyzed using Chi-square and P value where P value less than 0.05 is significant.

RESULTS

Prematurity is a highly significant risk factor predisposing to neonatal hyperbilirubinemia (p value < 0.001) as shown in (Table 1).

Table 1. Gestational Age

Gestational Age	Case	%	Control	%
Pre-Term✵	71	44%	47	18%
Full Term	89	56%	213	82%
Total	160		260	

✵ Prematurity is a significant risk factor (P<0.001).

The low birth weight [<2.5 kg] significantly predisposes to significant hyperbilirubinemia (p value < 0.001) as shown in (Table 2).

Table 2.Birth weight

Birth weight	Case	%	Control	%
<1.5 kg	21	13%	7	3%
1.5-2.499 kg✵	76	47%	78	30%
2.5-3.99 kg	62	39%	164	63%
≥ 4 kg	1	1%	11	4%
Total	160		260	

✵Low birth weight < 2.499 kg is significant (P<0.001).

The role of breast feeding in predisposing to significant hyperbilirubnemia is very highly significant (p value < 0.001) as shown in (Table 3).

Table 3. The type of feeding

Feeding type	Case	%	Control	%
Breast✵	64	40%	123	47.4%
Bottle	60	37%	39	15%
Mixed	36	23%	98	38%
Total	160		260	

✵Breast feeding is significant (P<0.001).

The use of oxytocin in induction of labor is very high significant predisposing factor to significant hyperbilirubinemia (p value < 0.001) as shown in(Table 4).

Table 4. The effect of the use of Oxytocin in the induction of labor

Oxytocin use	Case	%	Control	%
Yes✵	79	49%	173	67%
No	81	51%	87	33%
Total	160		260	

✵ Oxytocin use is significant (P<0.001).

The presence of cephalhematoma or bruises is high significantly predisposing to significant hyperbilirubinemia (p value = 0.015) as it is shown in (Table 5).

Table 5. The role of cephalhematoma and/or Bruises

Cephalhematoma and/or Bruises	Case	%	Control	%
Yes❋	12	8%	42	16%
No	148	92%	218	84%
Total	160		260	

❋cephalhematoma and/or Bruises is significant (P = 0.015).

The role of family history of significant hyperbilirubinemia in siblings is high significant predisposing factor (p value = 0.020) as shown in (Table 6).

Table 6. Family History of a sibling with significant neonatal hyperbilirubinemia

Family History	Case	%	Control	%
Yes❋	16	10%	10	4%
No	144	90%	250	96%
Total	160		260	

❋Family History of a sibling with significant neonatal hyperbilirubinemia is significant (P (P= 0.020).

Maternal age of more than 25 years is not significant as a predisposing factor of significant hyperbilirubinemia (p value = 0.063) as shown in (Table 7).

Table7. The effect of maternal age

Maternal Age	Case	%	Control	%
<25 yrs	79	49%	156	60%
≥25 yrs❋	81	51%	104	40%
Total	160		260	

❋Maternal Age ≥ 25 yrs are not significant (P = 0.063).

Among the breastfed babies, maternal age doesn't seem to be of significance in predisposition to significant hyperbilirubinemia as shown in (Table8) (p = 0.859). ≥ 25yrs 27 case breastfed 42% and 104 control 40%.

Table 8. The maternal age in neonate who were breastfed & controls

Maternal Age	Case (breast)	%	Control	%
<25 yrs	37	58%	156	60%
≥25 yrs❋	27	42%	104	40%
Total	64		260	

❋Among the breastfed babies maternal age ≥ 25 yrs is not significant (P = 0.859).

Low birth weight of less than 2.5 Kg is it is shown in (Table 9). (P ≤ 0.001) and it is very high significant predisposing factor as more clear in very low birth weight less than 1.5 Kg

Table 9. Birth weights of premature neonates with significant hyperbilirubinemia and controls

Birth weight	Case (premature)	%	Control (all)	%
<1.5 kg	20	30%	7	8%
1.5-2.499 kg❋	46	70%	78	92%
Total	66		85	

❋ *Birth weight < 2.499kg is significant (P = <0.001).*

DISCUSSION

This study investigated the effect of different risk factors other than hemolysis in predisposing to significant hyperbilirubinemia in neonates in Duhok city.

According to this study it is shown that prematurity (gestational age of < 37 weeks) is a significant factor predisposing to significant neonatal hyperbilirubinemia. This is due to immaturity of uridyldiphosphate glucuronyle transferase enzyme responsible for conjugation of bilirubin in the liver, decreased Y liganden receptors on the surface of hepatocytes and delayed and poor oral feeding which enhances the enterohepatic circulation. This agrees with that found by some other studies [5,6,7,8] where a study done in Italy by Bertini et-al showed that gestational age is not a significant predisposing risk factor [9].

The birth weight < 2.5 kg is found to be a significant predisposing risk factor for significant neonatal hyperbilirubinemia and this effect is found to be even more clear when birth weight < 1.5 kg. This is due to that most of these LBW neonates were premature and in those who are full terms, the high biliruben production is caused by the larger hematocrit due to in utero hypoxemia [7,8,10].

According to this study breast feeding is a significant predisposing risk factor to significant neonatal hyperbilirubinemia. This is due to delayed and impaired breast milk intake specially in mothers who are primigravida and not well experienced with breast feeding. This leads to weight loss and dehydration and lead to elevation of bilirubin level [10,11,12,13,14]. Also the breast milk contains an inhibitor which is a fatty acid that inhibits the function of UDPglucuronyl transferase enzyme and it contains the glucuronidase enzyme that converts the conjugated to uncongugated bilirubin and this enhances the enterohepatic circulation [15, 16, 17]. Similar results were found in many studies done allover the world[14,18,19,20,21].

In our study, we found a strong relation between significant neonatal hyperbilirubinemia and exposure to oxytocin. Oxytocin has a direct effect on neonatal bilirubin metabolism, and similar results were found in different studies done previously [21, 22,23,24,25].

According to this study, cephalhematoma and bruising are significant risk factors for hyperbilirubinemia. This is because the extravasated RBCs undergo hemolysis and produce indirect bilirubin [15,16,17].

Another risk factor for significant neonatal hyperbilirubenemia according to our study is a family history of previous sibling with significant neonatal hyperbilirubinemia. Similar results were found in previous studies[26,27].

Maternal age of > 25 years was not found to be significant as a predisposing factor of neonatal hyperbilirubinemia in this study. This is in contrast to some studies that considered it as a minor risk factor [4,26]. This is most probably because the effect of maternal age is blunted by the other risk factors that were found to be significant.

CONCLUSIONS

According to this study it is proved that prematurity, low birth weight, breast feeding, oxytocin exposure, cephalhematoma and bruises and also a family history of a previous sibling with significant hyperbilirubinemia are all significant risk factors for neonatal hyperbilirubinemia that mandates treatment.

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پوختە

فاکتەرێن ترسناك بۆ زێدەبوونا مادا بیلیرۆبین جورا (نەك ل ئەگەرا پرت بوونی خوینی) ل نوی زای ل دهۆکێ

پێشەکی: زەرك ئیك ژ ئاریشێن بەربەلاڤ تووشی نوی زای بیت .65٪ ل نوی زای توشی زەرکا کلینیکی بن ل حەفیتی ئیکی پاش ل دایك بوونی دەمی رێژا بیلیروبینی زیتر ل 5 ملیگرام /دی سی لیتر بیت.بیلیروبین ئیك ژ ب هیزترین دژی أوکیسدەنتە بەلی ل رەخەکا دی بیتە مادەکا ژەهراوی ئەگەرا رێژا وی گەلەك بلند بیت ئو ئاریشێن خراب بو میژی نوی زوی دروست کات

ئارمانج: ڤەکولینا فاکتەرێن ترسناك بۆ زێدەبوونا مادا بیلیرۆبین (نەك ل ئەگەرا پرت بوونی خوینی) ل نوی زای ل دهۆکێ

رێکێن ڤەکولینێ: دوو گروپ ل نوی زای هاتیە دەستنیشان کرن, گروپی ئیکی(کێس) پێکهاتبو ل (160) ل نوی زای توشی بلند بوونا رێژا بیلیروبینی بینە و هاتینە چارەسەرکرن ب رێکا ئامیرا فوتو یان ب گهورینا خوین, گروپی دووی (کونترول) پێکهاتبو ل (260) ل نوی زای بێ بلندبوونا بەرچاڤ یا بیلیبروبینی. هەردوو گروپ هاتیە ڤەکولین بەروارد ب هنەك فاکتەرا وەکو زاروك بوونی بەری تەمام کرنا ماوەی زگبری(37 حەفتی).جورا خارنێ , بکارئینانی اوکسی توسین بو ل دایك بوونی وبوونا ئاریشا بلندبوونا بیلیروبینی ل میژووی خیزان و دروست بوونا هیماتوما ل سەری .

ئەنجام: دیار بو کە زاروك بوونی بەری تەمام کرنا ماوەی زگبری(37 حەفتی) ل 71 کێسا(44٪) و ل 47 کونترولا (18٪) وسەنگی زاروکی کیمتر ل (2500 گرام) ل 76 کێسا(47٪) و ل 78 کونترولا(30٪) وشیردانی دایکی ل 64 کێسا(40٪) و ل 123 کونترولا(47٪) , وبکارئینانا أوکسی توسین ل 79 کێسا(49٪) و ل 173 کونترولا(67٪) ,دروست بوونا هیماتوما ل سەری ل 12 کێسا(8 ٪) و ل 42 کونترولا (16٪) ,بوونا ئاریشا بلندبوونا بیلیروبینی ل میژووی خیزانی ل 16 کێسا(10٪) و ل 10 کونترولا(4٪) .

دەرئەنجام: ل ئەنجامی ڤەکولینی دیارکەت کە هەمی فاکتەرێن دیارکری زاروك بوونی بەری تەمام کرنا ماوەی زگبری(37 حەفتی).جورا خارنێ ,بکارئینانی اوکسی توسین بو ل دایك بوونی وبوونا ئاریشا بلندبوونا بیلیروبینی ل میژووی خیزان و دروست بوونا هیماتوما ل سەری, پیوەندی ئاماری ب هیز هەنە دگەلّ بلندبوونا رێژا بیلیروبینی ل نوی زای.

الخلاصة

عوامل الخطورة لفرط البيليروبين المعتبرالغير التحليلي لدى حديثي الولادة في دهوك

خلفية: اليرقان الوليدي هي مشكلة مشتركة. خمسة وستين في المئة من الأطفال حديثي الولادة يصابون باليرقان السريرية مع مستوى البيليروبين أكثر من 5 ملغ / دل خلال الأسبوع الأول من الحياة. البيليروبين هو أحد مضادات الأكسدة القوية التي يمكن أن تساعد الأطفال حديثي الولادة الذين تعاني من عجز في معظم المواد المضادة للأكسدة. فرط بيليروبين الدم يمكن أيضا أن تكون سامة لديهم مستويات عالية مما أدى إلى اعتلال الدماغ. تحديد الأطفال الرضع في خطر الاصابة فرط بيليروبين الدم الشديد وحديثي الولادة اليرقان هي المشكلة التي واجهت الأطباء منذ حالة من اليرقان الوليدي في البداية تم الاعتراف منذ أكثر من 100 سنة.

الهدف: لتقييم عوامل مخاطر مهمة للفرط بيليروبين الدم الوليدي كبيرة في دهوك، اقليم كوردستان العراق.

طرق البحث: وشملت الدراسة 160 حالة من فرط بيليروبين الدم الوليدي غير الانحلالي التي تحتاج إلى إدارة مع العلاج بالضوء أو تبادل نقل الدم و 260 الضوابط الذين كانوا حديثي الولادة دون فرط بيليروبين الدم كبيرة. تمت دراسة المجموعتين من حيث النضج ونوع التغذية، واستخدام الأوكسيتوسين للحث على العمل، وجود ورم دموي رأسي وأو كدمات ووجود تاريخ عائلي لأحد الأخوة الذي كان **فرط البيليروبين** كبيرة وكانت النتائج تحليلها إحصائيا باستخدام اختبار مربع كاي حيث P أقل من 0.05 مهم.

النتائج: كان الخداج الحالي في 71 حالة (44٪) وP47 (18٪) controls <0.001، الوزن الميلاد كان حاضرا في 76 حالة <2500 جرام (47٪) وP78 (30٪) control <0.001، كان الرضاعة الطبيعية الحاضر في 64 حالة (40٪) و 123 الضوابط (47٪) P <0.001، وكان يستخدم الأوكسيتوسين في 79 حالة (49٪) و 173 الضوابط (67٪) P <0.001، وكان ورم دموي رأسي موجودة في 12 حالة (8٪) و 42 ضوابط (16٪) P = 0.015، تاريخ عائلي من أخ كبير مع فرط بيليروبين الدم كان حاضرا في 16 حالة (10٪) و 10 عناصر التحكم (4٪) P = 0.02

المناقشة والاستنتاج: من خلال التحليل الإحصائي تبين أن الخداج، وانخفاض الوزن عند الولادة (<2.5 كجم)، والرضاعة الطبيعية، واستخدام الأوكسيتوسين للحث على العمل، وجود ورم دموي رأسي و / أو كدمات والأخوة مع التاريخ من فرط بيليروبين الدم كبيرة كلها العوامل المؤهبة كبير كما يتفق مع العديد من الدراسات التي أجريت في كافة أنحاء العالم

ROLE OF OSTEOPONTIN AND OXIDATIVE STRESS IN CORONARY ARTERY ATHEROSCLEROSIS AND ISCHEMIC HEART FAILURE IN TYPE 2 DIABETES MELLITUS

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ABSTRACT

Background and Objectives There is now a consensus that atherosclerosis represents a state of heightened oxidative stress characterized by lipid and protein oxidation in the vascular wall. The objective of this study was to measure levels of these mediators in the systemic circulation as biomarkers for potential use in risk assessment in patients with type 2 diabetes mellitus.

Methods :This study involved 108 patients with type 2 diabetes mellitus admitted to the angiography department for assessment of coronary artery diseases, divided into 3 groups, 66 patients with positive angiography results, 25 with ischemic heart failure, 17 with negative angio results, we compared them with 30 non diabetic subjects with negative angiography results, assessment of plasma osteopontin, serum soluble receptors for advanced glycation end products and total antioxidants capacity were done by ELISA technique.

Results: serum total antioxidants capacity in diabetic patients with positive angio and diabetic with ischemic heart failure were significantly lower compared with other studied groups (non-diabetic controls and diabetic with negative angio), mean serum soluble receptors for advanced glycation end products level was lowest among diabetics with positive angio and ischemic heart failure and highest in the non-diabetic controls, patients with atherosclerosis proved by angiography (diabetic with positive angio and diabetics with positive angio and ischemic heart failure) showed appreciable and significantly higher levels of plasma osteopontin levels compared with other study groups without atherosclerosis (non-diabetic control and diabetic with negative angio).

Conclusion: Measurement of osteopontin and oxidative stress biomarkers are of value for diagnosis of macrovascular complications in patients with type 2 diabetes mellitus.

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Key words: Osteopontin, sRAGE, Total antioxidant, Atherosclerosis, T2DM.

Diabetes mellitus (DM) is a major health problem throughout the world[1]. Early diagnosis of diabetes aims to prevent long term complications. Because cardiovascular disease is the main complication of Type 2 diabetes mellitus (T2DM), recent studies have investigated the capability of new criteria to predict these complications. The association of hyperglycemia and cardiovascular diseases is a crucial one on which to test the validity of the new criteria[2].

Oxidative stress, through the production of reactive oxygen species (ROS), has been implicated in the progression of long-term diabetes complications, including microvascular and macrovascular dysfunction. Excess nourishment and a sedentary lifestyle leads to glucose and fatty acid overload, reaction of glucose with plasma proteins forms advanced glycation end products (AGEs)[3,4]. AGEs bind with receptors on the surface of endothelial cells lining blood vessels.

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Receptors for AGEs (RAGE) are expressed in many different tissues and cell types, including endothelial cells, vascular smooth muscle cells and macrophages. The binding of AGEs to RAGE leads to the intracellular generation of ROS[3].

Osteopontin (OPN) is an extracellular matrix protein that mediates cell-cell and cell-matrix interactions. OPN was first isolated from bony matrix[5], OPN is highly expressed in human atherosclerotic lesions and is not only a marker of inflammation but also an active player in the progression of atherosclerosis[6]. OPN stimulates proliferation of smooth muscle cells, migration of endothelial cell and recruitment of macrophages, all of which are directly related to atherosclerosis process[7]. Plasma OPN levels are associated with the presence and extent of coronary artery disease[5]. Recent reports show that high glucose levels stimulate OPN expression through protein kinase C-dependent pathway as well as hexosamine pathways in cultured rat aortic smooth muscle cells[8]. Furthermore, OPN expression has been shown to be up-regulated in the vascular wall of diabetic patients and diabetic animal models, which might be induced by high glucose and AGEs9, in addition OPN deficiency has been shown to result in reduced atherosclerotic lesion areas [10, 11].

The aims of the present study were to evaluate the roles of osteopontin and oxidative stress in pathogenesis of atherosclerosis & ischemic heart failure (IHF) in patients with T2DM.

METHODS

Participants of the current research included one hundred-thirty eight patients (57 males and 81 females), admitted to the cardiac catheterization and angiography Department in Azady Teaching Hospital.

Patients (n=108) were divided into two major groups:

1. The first group comprised of one hundred-eight patients with type 2DM who had been referred for assessment of coronary artery disease, the diagnosis of diabetes was made from a previous diagnosis and drug history. This group was subdivided into three subgroups, as follows;

a. Sixty-six (28 males and 38 females) patients angiographically proved to have coronary artery disease (CAD) and with an age range of 32-80 years (mean ± SE of 55.6 ± 1.22), the inclusion criteria were diabetic patients with angiographically documented CAD.

b. Twenty-five (16 males and 9 females) patients angiographically proved to have CAD and with an age range of 42-77 years (mean ± SE of 60.4 ±1.65) and they were diagnosed as having ischemic heart failure on the basis of history of ischemic heart diseases, ischemic ECG changes, history of anti-ischemic and heart failure drug intake, and based on ejection fraction measurements by echocardiography of 0.4512 or less, all the patients were receiving standard therapy at time of enrolment.

c. Seventeen (4 males and 13 females) patients with negative angiography results, they were regarded as positive control and with an age range of 43-65 years, (mean ± SE of 52.9 ± 1.89).

2. The second group included thirty (9 males and 21 females) non-diabetic individuals they served as negative control group their ages ranged from 24-72 years (mean ± SE 47.7 ± 2.02). Patients were recruited in catheterization department after undergoing coronary angiography for suspected CAD. Patients were only considered if invasive examination and echocardiography excluded CAD as well as normal systolic and diastolic cardiac functions with normal results on routine laboratory testing [13]. Informed consent was obtained from each subject before study entry and the study was approved by the local research ethics committee.

Pre-tested questionnaire was designed to obtain information on age, gender, height, weight, smoking history, past medical history, history of myocardial infarction, systemic hypertension, diabetes mellitus, family history of diabetes, coronary artery disease and hypertension.

Blood samples were withdrawn from a suitable forearm vein, and used for assessment of glycated hemoglobin (HbA1c) and measurement of biochemical markers. All patients underwent echocardiography examination by cardiologists one day before coronary artery angiography study. Non-invasive color Doppler echocardiography was used for assessment of ventricular wall function, and measurement of ejection fraction%.

Serum total antioxidants capacity was measured by calorimetric microplate assay using Oxford Biomedical Research ELISA kit, Osteopontin was measured by invitro enzyme-linked immunosorbent assay for human osteopontin (Ray Biotech) ELISA kit, human sRAGE ELISA was measured by a sandwich enzyme immunoassay (MyBioSources), hemoglobin A1c measured in human blood using fluorescence immunoassay í-CHROMA.

Statistical analyses were done using SPSS version 13 computer software (Statistical Package for Social Sciences). We assumed the level of statistical significance at P <0.05.

RESULTS

Table (1) illustrates that serum TAC in diabetic patients with positive angio and diabetic with IHF were significantly lower compared with other studied groups (non-diabetic controls and diabetic with negative angio). Moreover, patients with positive angio and IHF showed significantly lower serum TAC compared with diabetics with positive angio (868.5 Vs 1170.5, P= 0.035).

Table1. Measurement of Serum Total Antioxidants Capacity in the Studied Groups

Studied groups	Controls	Patients		ANOVA
	Negative controls (non-diabetic-ve angiography)	Positive control (DM with -ve angiography)	DM with +ve angiography	DM with +ve angiography and IHF	P-value
Variable	N=30	N=17	N=66	N=25	
TAC (µmol/L) Mean ± SE	1784.7 ± 220.84	1521.4 ± 60.48	1170.5 ± 26.04	868.5 ± 66.09	<0.001
P–value of LSD test for difference in mean between groups	Non-diabetic controls (-ve angio) X DM with +ve angio				<0.001
	Non-diabetic controls (-ve angio) X DM with +ve angio and IHF				<0.001
	Positive control (DM with -ve angio) X DM with +ve angio				=0.035
	Positive control (DM with -ve angio) X DM with +ve angio and IHF				<0.001
	DM with +ve angio X DM with +ve angio and IHF				=0.035

The differences observed in mean sRAGE levels between all the studied groups were statistically significant (P=0.006). Mean sRAGE level was lowest among diabetics with positive angio and IHF (393.9 pg/ml) and highest in the non-diabetic controls (564.7 pg/ml) (Table 2). All patient groups showed significantly lower mean sRAGE levels compared with non-diabetic controls. (Table 3) shows the results obtained from plasma Osteopontin measurements in the studied groups. Patients with atherosclerosis proved by angiography (diabetic with positive angio and diabetics with positive angio and IHF) showed appreciable and significantly

higher levels of plasma Osteopontin levels compared with other study groups without atherosclerosis (non-diabetic control and diabetic with negative angio) (Figure 1).

Table 2. Measurements of serum soluble receptors for advanced glycation end products (sRAGE) in the studied groups

Studied groups	Controls	Patients		ANOVA
	Negative controls (non-diabetic-ve angiography)	Positive control (DM with -ve angiography)	DM with +ve angiography and IHF	P-value
Variable	N=20	N=12	N=23	N=10	
sRAGE (pg/ml)					
Mean ± SE	564.7 ± 43.35	474.5 ± 51.33	403.5 ± 21.67	393.9 ± 35.48	=0.006
P-value of LSD test for difference in mean between:					
			Non-diabetic controls (-ve angio) X DM with +ve angio		=0.001
			Non-diabetic controls (-ve angio) X DM with +ve angio and IHF		=0.005

Table 3. Measurements of plasma Osteopontin in the studied groups

Groups	Controls	Patients		ANOVA P-value
	Negative controls (non-diabetic-ve angiography)	Positive control (DM with -ve angiography)	DM with +ve angiography and IHF	
Variable	N=20	N=12	N=23	N=10	
Osteopontin(pg/ml)					
Mean ± SE	429.2 ± 52.96	443.3 ± 60.98	1296 ± 108.12	1523.2 ± 210.09	<0.001
P-value of LSD test for the difference in mean between groups	Non-diabetic controls (-ve angio) X DM with +ve angio				<0.001
	Non-diabetic controls (-ve angio) X DM with +ve angio and IHF				<0.001
	Positive control (DM with -ve angio) X DM with +ve angio				<0.001
	Positive control (DM with -ve angio) X DM with +ve angio and IHF				<0.001

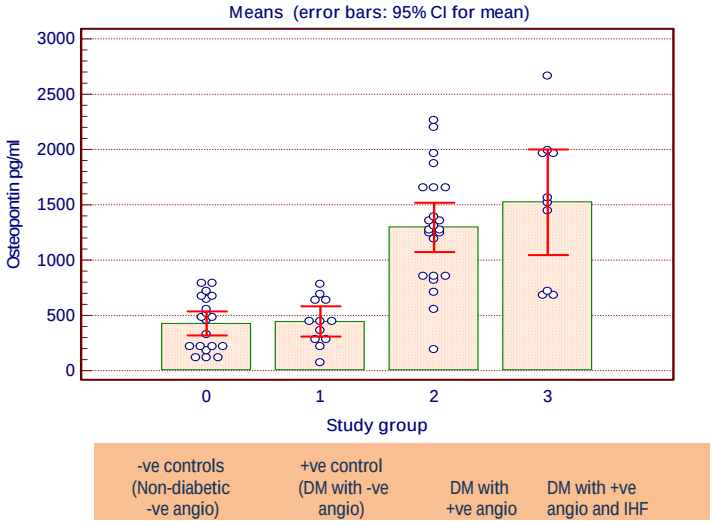

Figure 1. Serum OPN level (pg/ml) in the studied groups.

Correlation between selected parameters in the studied groups:

There was a statistically significant positive correlation was found between serum RAGE and FBS (r=0.547, P=0.013) in non-diabetic patients with negative angio (negative control group). In diabetics with positive angio, plasma OPN level was significantly positively correlated with BMI (r=0.495, P=0.016).

In diabetic patients with positive angio and IHF, TAC significantly negatively correlated with age (r=-o.478, P=0.016). Interestingly statistically significant negative correlation was observed between OPN level and Ejection fraction% (EF%) (r=-0.647, P=0.043) (Figure 2).

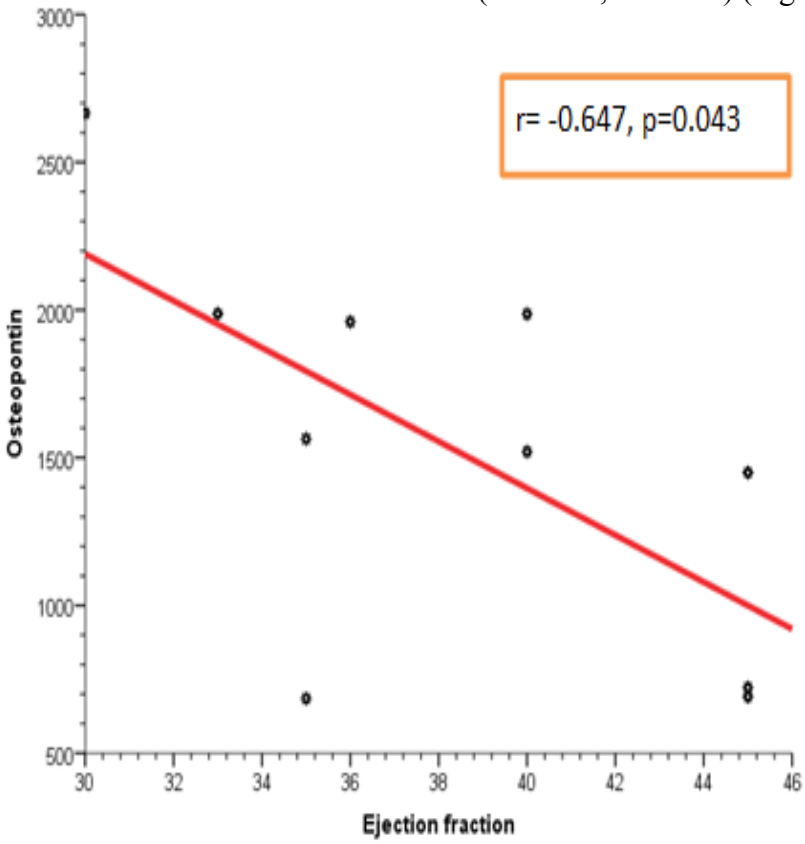

Figure 2. Scatter diagram with fitted regression line showing the correlation between OPN and EF% among DM with positive angio and IHF

ROC Area and validity for selected parameters when used to predict diabetic with positive angio differentiating them from positive diabetic controls:

OPN and TAC were of high validity (AUC = 0.942 and 0.885, P<0.001) respectively, and had statistically significant role in predicting atherosclerosis among diabetics.

OPN optimum (typical) cut-off value and highest specificity is ≥799.4 pg/ml which yields a sensitivity of 87.0% and specificity of 100%. Testing positive at this cut-off value may establish the diagnosis of atherosclerosis in diabetic patients with 100% confidence. In the same context, testing negative might exclude the diagnosis of atherosclerosis in diabetic patients with (98.6%) confidence (Table4).

Table 4. Validity parameters for selected tests when used to predict diabetics with positive angio (PPV) differentiating them from positive diabetic controls (NPV)

Parameters	Sensitivity	Specificity	Accuracy	PPV at pre-test Probability = 50%	PPV at pre-test Probability = 90%	NPV at pre-test Probability = 10%
Positive if ≥ cut-off value Osteopontin (pg/ml)						
132.2 (highest sensitivity)	**100.0**	**8.3**	**68.6**	**52.2**	**90.8**	**100.0**
799.4 (highest specificity & typical value)	**87.0**	**100.0**	**91.5**	**100.0**	**100.0**	**98.6**
ROC area=0.942 , P≤ 0.001						
Positive if ≤ cut-off value Total antioxidants capacity µmol/L						
1048.8 (highest specificity)	**45.0**	**100.0**	**65.6**	**100.0**	**100.0**	**94.2**
1454.0 (highest sensitivity & typical value)	**100.0**	**58.3**	**84.4**	**70.6**	**95.6**	**100.0**
ROC area=0.885 , P≤ 0.001						

ROC Area and Validity for Selected Parameters when used to Predict Diabetics with IHF Differentiating Them from Diabetics with Positive Angio only:

TAC was the only parameter, which had a high validity in predicting heart failure among diabetics with positive angio (AUC=0.87, P=0.001), in addition to EF (AUC=1, P<0.001). The optimum (typical) cut-off value of TAC associated with highest sensitivity is (1014.3µmol/L) may help the diagnosis of IHF in diabetic patients with positive angio with (96.8%) confidence. In the same context, testing negative might exclude IHF in diabetic patients (100%) confidence (Table 5).

Table 5. Validity parameters for selected tests when used to predict diabetics with IHF differentiating them from diabetics with positive angio only

Parameters	Sensitivity	Specificity	Accuracy	PPV at pre-test probability = 50%	PPV at pre-test probability = 90%	NPV at pre-test Probability = 10%
Positive if ≥ cut-off value Total antioxidant capacity Mmol/L						
454.1 (highest specificity)	**20.0**	**100.0**	**73.3**	**100.0**	**100.0**	**91.8**
1014.3 (highest sensitivity & typical value)	**100.0**	**70.0**	**80.0**	**76.9**	**96.8**	**100.0**
ROC area=0.87 , P≤ 0.001						

DISCUSSION

In this study serum TAC of the diabetic patients with positive angio and diabetic with IHF were found to be significantly lower compared with non-diabetic controls and diabetic with negative angio. Gul et al.[14] (2010) found that among patients with cardiovascular diseases, patients with MI had low TAC levels. They considered that free radicals generated by hyperglycemia-dependent endothelial dysfunction is counterbalanced by antioxidants[15], Valabhji et al.[16] (2001), reported that TAC was reduced in diabetic subjects compared with non-diabetic subjects (P < 0.001).

Mean RAGE level was found to be statistically lowest among diabetics with positive angio and IHF comparing with the non-diabetic controls. Kiuchi[17] (2001)

found higher mean serum AGE concentrations ($p < 0.0125$) in type 2 diabetic patients with obstructive coronary artery disease (CAD) than in patients without it, and higher than in non-diabetic patients with and without obstructive CAD, this finding indicates that serum AGE concentrations may be associated with long term uncontrolled glycemic state and reflect the severity of coronary arteriosclerosis in T2DM patients. Moreover, a statistically significant positive correlation was found between serum RAGE and fasting blood sugar ($r=0.547$, $P=0.013$) in the present work. Negrean et al.[18] (2007) reported that T2DM patients have significantly higher serum AGE concentrations than do healthy control subjects because hyperglycemia and oxidative stress both contribute to their accumulation. Elevated serum AGEs are associated with increased CAD in type 2 diabetic subjects. AGEs may be associated with atherosclerosis in a number of ways, including increased endothelial dysfunction, elevated vascular LDL, increased plaque destabilization, neointimal proliferation, and inhibited vascular repair after injury[19].

Patients with atherosclerosis proved by angiography (diabetic with positive angio and diabetics with positive angio and IHF) showed appreciable and significantly higher levels of plasma OPN levels compared with other studied groups without atherosclerosis (non-diabetic control and diabetic with negative angio), the results of this study are in agreement with that reported by Minoretti et al.[20] (2006) who have shown a statistically significant trend towards higher cardiovascular event rates in those patients with higher OPN, these results were also in harmony with that of Tanaka et al.[21] (2006), in which high plasma OPN levels reported in patients with coronary artery disease (CAD) and to correlate with the severity of CAD. Similar findings were also observed by Yan et al.[8] (2010), who found that patients with OPN concentrations greater than the median value had a higher incidence of CAD than those with OPN levels below the medium value.

The levels of plasma OPN were significantly elevated in patients with IHF as compared with non-diabetic control subjects ($P<0.001$). Frey et al. [22] (2010) reported that median OPN plasma level in the control sample was significantly lower than patients with systolic heart failure ($p<0.01$). However, it appears that OPN is associated with the presence and the extent of cardiovascular diseases. OPN can serve as a chemo-attractant for a number of cell types, thus, production of OPN at the sites of endothelium injury may modulate the proliferation, migration, and accumulation of endothelial and vascular smooth muscle cells, thereby promoting vascular repair, but also perhaps initiating vascular calcification[23].

In diabetics with positive angio group, plasma OPN level was significantly positively correlated with BMI ($r=0.495$, $P=0.016$), similar findings was observed by Gürsoy et al. [22] (2010) they found that OPN levels of obese patients were significantly higher than its level in non-obese controls. In diabetic patients with positive angio and IHF, the TAC significantly negatively correlated with age ($r=-o.478$, $P=0.016$), the finding was in agreement with the previous study, that found TAC value becomes decreased with the increase in age[22]. Interestingly statistically significant negative correlation was observed between OPN level and EF% ($r=-0.647$, $P=0.043$, Figure 2), our finding was in consistence to the findings of Satoh et al. [25] (2005) they found that OPN mRNA were negatively correlated with left ventricular EF.

It was found that, OPN and TAC were had a high validity (AUC= 0.942 and 0.885, $P<0.001$) respectively, and had statistically a significant role in predicting angiographic evidence of atherosclerosis among diabetics. Valabhji et al.[16] (2001) reported that a decrease in serum TAC

level was entered as a predictor of CAD, and the effects on its predictive value of adding other explanatory variables in bivariate analyses were assessed. The power of TAC was independent of many of the traditional risk factors.

The capacity of OPN when used to differentiate diabetics with positive angio and diabetic with positive angio and IHF from non-diabetic and diabetic controls was statistically significant, Ömer et al.[26] (2009) studied ROC analysis for the role of OPN in coronary artery calcification (CAC), the AUC for identification of CAC was greatest for OPN (p=0.004).

CONCLUSION

It is concluded that serum TAC is decreased in diabetic patients with positive angio and diabetic with IHF, suggesting the presence of oxidative stress, and that change in TAC seems to be influenced by diabetes severity. OPN and RAGE measurements may be important and useful biomarkers in estimating the severity of diabetic complications including CAD and is a probable indication for the administration of antioxidants in the management of the disease.

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پوختە

رولێ وستیوپونتین وئەرکی اوکساندنێ ل رەقکرنا خوینبەرێن دلی و نەخوشیا لاوازی دلی و نەخوشیا شەکرێ ل جورێ دووێ

پیشە کی و ئارمانج: پاشگویی و مەرەم ,دڤی دەمیدا وەك هەڤن ل سەر رەق بونا خوینبەرا وەکی کێسێن اکساندنەکا زور ئەوا بەر چاڤ ب اوکساندنا پروتیناتا ل سەر دیوارێن ملولێت خوینێ. ئارمانج ژ ڤێ ڤەکولینێ بو هەلسەنگاندنا ئاستێ ناوەندی د سیستەمێ زڤرینا خوینێدا و نیشانێت زیندو بو بکار ئینانا پێتڤی د هەلسەنگاندنا مەترسیێن ل نەخوشیێن توشی ئشا شەکرێ بین ژ جورێ دووێ ئەوێت نەپێتڤی ب ئەنسولینێ بو چارەسەریێ.

رێکێن ڤەکولینێ: ئەڤ ڤە کولینە 108 نەخوشێن شەکرێ ژ جورێ دووێ ب خو ڤەدگریت و ئەڤێن دبەنە پشکا قەستەرێ بو هەلسەنگاندنا نەخوشیێن خوینبەرێن تاجی ئەوێن هاتینە دابەش کرن ل سەر سێ گروپا ژ وان 66 نەخوش ئەنجامێن وێنەییێن ملولا موجەبن(گروپێ 1) و25 نەخوش ئەنجامێن وێنەییێن ملولا موجەبن و د نالن ژ نەخوشیا لاوازیا دلی IHF (گروپێ 2) و 17 نەخوش ئەنجامێن ملولا سالب (گروپێ 3) جیاوازی د ئەنجامێن گروپێن نەخوشێن شەکرێ دگەل 30 نەتوش بوین نەخوشیا شەکرێ و ئەنجامێن ملولێن خوینێ ل نك وان سالبن ان کو نەتوش بوی ب نەخوشیا رەق بونا خوینبەرا (گروپێ 4) هەر وەسا ب هەژمارتنا ئاستێن شیێانێن گروپێن دژ ب اکساندنا RAGE د ناف سیرەم و استیوپنتین د پلازمایدا هەروەسا پێشوازیکەرێتTAC , بو زانین کو هەمی رێکێن هەژمارتنی تێنە ئەنجامدان ب ئا میرێ ELISA.

ئەنجام: هەلسانگاندنا شییانێن گروپێن دژە اوکساندنا بو گروپێن 1و 2 جییاوازیین وانا بێت بو واتە کێمترن ب جییاواز دگەل گروپێن 3 و 4 بو ب هێز کرنا سەلمینەرا وەستان اوکساندنێ.ئەڤا کو دوپات دکەت کو چەند تێکراییا دژ اوکساندنێ کێم دبیت نەساخ کاریگەرتر دبیت لسەر لەشی .بەلێ هندی کو ئاستێ پێشوازیکەرێن RAGEدناف سیرەمیدا ئامارێن گروپێ 1 و 2 کێمتر دبن جیاواز دگەل گروپێ 3 و4 و هندەك ئاستێ استیوپونتین دناف پلازمایدا جیاوازن بەلگەییێن ئاماری بلندترن د گروپێن 1 و2 جیاواز د گروپێن 3 و 4 دا.

دەرئە نجام : ئەنجامێ پیڤانا نیشانێت زیندەیی وەکی شییانێت گروپێ دژ اوکساندنا استیوپونتین ئو هەر وەساRAGEدناف خوینێدا خودان بهایەکێ گرنگن بو دیف چونا نەخوشیا شەکرێ ژ جورێ 2 و ئەوێن توشی سەربارکێن ملولێن مەزن دبن.

الخلاصة

دور الاوستيوبونتين والإجهاد التأكسدي في تصلب الشريان التاجي ومرض عجز القلب الوعائي في مرضى السكري من النوع الثاني

الخلفية والأهداف هناك الآن إجماع على أن تصلب الشرايين يمثل حالة من الاكسدة المتزايدة التي تتميز أكسدة الدهون والبروتين في جدار الأوعية الدموية. وكان الهدف من هذه الدراسة لقياس مستويات هذه الوسطاء في جهاز الدورة الدموية والمؤشرات الحيوية لاستخدامها المحتمل في تقييم المخاطر في المرضى الذين يعانون من مرض السكر من النوع الثاني الغير معتمد على الانسولين في العلاج .

طرائق البحث: وشملت هذه الدراسة 108 مريض السكر النوع 2 والذين ادخلوا الى قسم القسطرة لتقييم أمراض الشريان التاجي ، والذين قسموا الى 3 مجموعات ،منهم 66 مريضا كانت نتائج التصوير الوعائي موجب(المجموعة الاولى) ، و 25 مريض يعانون من عجز القلب الناتج عن نقص التغذية الدموية) IHFالمجموعة الثانية) و 17مريض وكانت نتائج التصوير الوعائي سالب (المجموعة الثالثة) وتم مقارنة نتائج مجموعة مرضى السكري مع 30 من غير المصابين بمرض السكر ونتائج التصوير الوعائي لديهم سالبة اي لايعانون من تصلب الشرايين(المجموعة الرابعة) كذلك تم قياس مستويات قدرة مجموعة المواد المضادة للأكسدة (TAC) في المصل و Osteopontin في البلازما وكذلك مستقبلات AGEs في مصل الدم علما بان جميع طرق القياس تمت بواسطة تقنية.ELISA

النتائج : قيم قدرة مجموعة مضادات الاكسدة للمجموعة الاولى والثانية كانت ذات فروق معنوية أقل مقارنة مع المجموعتين الثالثة والرابعة مما يعزز نظرية الاجهاد التأكسدي التي تؤكد كلما انخفض معدل مضادات الأكسدة كلما كانت شدة المرض اكثر تأثيرا على الجسم . اما مستويات مستقبلات ال RAGE في المصل كانت احصائيا أدنى في المجموعتين الاولى والثانية مقارنة مع المجموعتين الثالثة والرابعة اما مستويات ال Osteopontin في البلازما كانت ذات فروق ذات دلالة معنوية احصائيا اعلى في المجموعتين الاولى والثانية مقارنة مع المجموعتين الثالثة والرابعة.

الخاتمة : نتائج قياس المؤشرات الحيوية مثل قدرة مجموعة مضادة للأكسدة و Osteopontin وكذلك ال RAGE في الدم هي ذات قيمة مهمة لمتابعة مرضى السكري النوع الثاني والذين يعانون من مضاعفات الأوعية الكبيرة.

THE EFFECT OF SOLUBLE BETA GLUCAN OF SACCHAROMYCES CEREVISIAE ON GROWTH OF AMN3 TUMOR CELL LINE IN VIVO

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ABSTRACT

Background and objectives This study was designed to evaluate the anticancer effects of the soluble beta glucan (ß-glucan) local and commercial extracts of the Saccharomyces cerevisiae on cancer cell line AMN3 in vivo.
Methods : In vivo study was performed on cancer cell line murine mammary adenocarcinoma (AMN-3) cell line and the median lethal dose (LD50) of β-glucan extract through SC administration in BALB/c mice.
Results: BALB/c mice showed no LD50 to all the concentration of ß-glucan (200, 400, 600, 800, 1000 µg/ml).The effect of the ß-glucan extract on relative tumor volume (RTV) for three weeks (that treated after 24hrs of tumor implantation) , and also for one month (after tumor implantation) had the best effect when compared with commercial extract. RTV was not denoted in mice groups that treated before tumor cell implantation and gave the best protocol for cancer treatment. The period of three weeks show that all groups have significant inhibition ($P<0.05$) percentage (38.6,42.6,50.14, and 51.67)% for local extract, (35.22, 42.17, 43.5 and 45.5)% for commercial , so the local and commercial extracts gradually increase the tumour growth inhibition (TGI) depends to the time, except in commercial group showed decrease in TGI (38.3%) at fifteenth day ,also the local extract showed decrease in TGI at the periods of sixth (35.8%) days, reveals tumour growth inhibition of local and commercial extracts for one month .
Conclusions: The soluble β-glucan of Saccharomyces cerevisiae (local & commercial extracts) had a great potent cytotoxic effect against tumor cells line AMN-3 in mice.

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Key words: ß- glucan (β-G) , AMN-3(Adeno mammary carcinoma) , TGI (Tumor growth inhibition) , RTV(Relative Tumor Volume)

Cancer is one of the leading causes of death in the world. The main cause is that they damage immune systems in tumor treatment. So, it is necessary to develop novel anti-tumor agents with administrating immunity potential. The polysaccharides have attracted more attention recently in the biochemical and medical fields because of their anti-tumor and immunomodulating properties [1]. Some polysaccharides extracted in medicines laboratory have been reported to possess anticancer activities [2].

Recent developments of modern techniques of targeted tumor cell elimination include Immunotherapy, which also called biological therapy, that uses the body's own immune system to fight cancer [3,4], and gene therapy, as a new trials to treat cancer [3]. However, there is a continuing need for development of new anticancer drugs, drug combinations and chemotherapy strategies, by methodical and scientific exploration of enormous pool of synthetic, biological and natural products [5]. A safe and effective cancer treatment has been the goal of scientists for many decades. Such a technique must be selective in destroying the cancer cells without irreversibly damaging normal cells [6].Beta glucan is a scientifically proven biological defence modifier (BDM) that nutritionally potentiates and modulates the

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immune response. through immune response potentiation and modulation, in many instances various therapeutic healing effects generated by the immune cells. For many years glucan have been investigated for these immune enhancing properties [7,8]. β-glucans are polysaccharides of D-glucose monomers linked by β-glycosidic bonds. β-glucans are a diverse group of molecules that can vary with respect to molecular mass, solubility, viscosity, and three-dimensional configuration. They occur most commonly as cellulose in plants, the bran of cereal grains, the cell wall of baker's yeast, certain fungi, mushrooms and bacteria [9]. Immunotherapy can lead to effective immune recognition and/or elimination of tumors, both CD4+ and CD8+ T cells have been identified as components of breast tumor infiltrating lymphocytes despite these specific immune responses, tumor cells manage to evade detection and/or destruction [7]. Recent advances in tumor immunology have provided a more complete understanding of the interaction of tumors with the immune system, and have delineated the diverse mechanisms by which tumor cells circumvent the immune response [10], Dendritic Cells (DCs) have been recognized as important mediators of immune response. They are specialized antigen-presenting cells that are highly potent in their presentation of antigen to naïve or quiescent CD4+ and CD8+ T cells. They capture, process, and present antigens in combination with Major Histocompatibility Complex (MHC) class I and II molecules, activating specific Cytotoxic T lymphocytes (CTLs). This ability to stimulate CTLs directly and effectively makes DCs ideal targets to exploit for manipulation of the immune system for cancer immunotherapy purposes [11]. Programmed cell death, is modulated by anti-apoptotic and proapoptotic effectors, which involves a large number of proteins. The proapoptotic and anti-apoptotic members of the Bcl-2 family act as a rheostat in regulating programmed cell death and are considered as targets of anticancer therapy [12]. The ratio of death antagonists Bcl-2 to agonists Bax determines whether a cell will respond to an apoptotic stimulus. Down-regulation of the death suppressor Bcl-2 could inhibit tumor growth via promoting programmed cell death [13]. It has been proven that Bax promotes apoptosis whereas Bcl-2 suppresses apoptosis 7,[14].

The general aim of the study was to evaluate the anti-tumor activities of the soluble β-glucan extracted from S. cerevisiae against tumor cells line AMN-3 in mice in vivo; using the following parameters:

1 - Determination the lethal dose (LD50) of the extract β-glucan of Saccharomyces cerevisiae in normal mice to evaluate the therapeutic doses.

2 - Determination the effect of β-glucan local and commercial extracts on the growth of transplanted tumor in mice.

METHODS

Ahmed-Mohammed-Nahi-2003 (AMN-3 cell line)

The cell line was supplied by Tissue Culture Unit / Iraqi Centre for Cancer and Medical Genetics Research (ICCMGR), Baghdad, Iraq (passage number 162) maintained in RPMI- 1640. The origin and description of this cell line was first mentioned by Al-Shamery [15]. The specimen was taken from murine mammary adenocarcinoma.

Experimental animals

Seventy female inbred BALB/C mice, aged 6-8 weeks and weight range (20-25gm), supplied from animal house / (ICCMGR) were used .They were housed and maintained in a conventional animal facility, with controlled conditions of temperature (20 ± 5ºC) and (10 and 14 hours of light and dark respectively). The animals were fed on special formula food pellets and given water on an ad libitum. Throughout the experiments, each ten

animals were housed in a plastic cage containing hard-wood chip as bedding. The bedding was changed weekly to ensure a clean environment.

Preparation of soluble beta glucan extract from S. cerevisiae (local extract): According to the [16,17]the baker's yeast β-glucan material was obtained from the Market.

A – Preparation of particulate β-glucan
Saccharomyces cerevisiae was processed from common, active dry yeast (300)g then added to one litre 0.1 mol of NaOH and stirred for 30 min at 60 °C. The material was then heated to 115 °C at 8.5 pressure /inch for 45 minute and then allowed to settle for 72 h. The sediment was resuspended and washed in D.W. by centrifugation 350 xg for 20 min. The alkali insoluble solids were combined with 0.1 mol of 1L of acetic acid and heated to 85 °C for 1 h, then allowed to settle at 38 °C. The acid insoluble solids were drawn off and centrifuged as above. The compacted solid material was mixed with 3% H2O2 and refrigerated for 3h with periodic mixing. Then the material centrifuged and the pellet washed twice with D.W. followed by two washes in 100% acetone. The harvested solid material was dispersed on drying trays and dried under vacuum at 38 °C for 2h in the presence of Ca2SO4, and then further dried overnight under vacuum at room temperature. This procedure yielded a bright yellow powder.

B- Preparation of soluble local β- glucan extract.
The particulate β-glucan was phosphorylated by dissolving 4g of β-glucan powder in 200ml of Dimethyl sulfoxide Me2SO containing 72g of urea. With stirrer, about 40ml of phosphoric acid 85% H3PO4 was added drop wise slowly to the above solution at ambient temperature. Then the solution was heated to 100 ◦C, and the reaction was carried out for 6h with stirring. A crystalline precipitate (presumed ammonium phosphate) formed at 1–2h of reaction. Following heating, the reaction mixture was cooled to ambient temperature and diluted in distilled-water to form a yellow bright solution. Finally, the resulting phosphate derivative was dialyzed (3000 – 5000)µm Millipore in size against double D.W. for seven days to remove endotoxin (includingMe2SO,H3PO4 and salt). The total concentration of carbohydrates present in the extract was determined according to the method use by Dubois et al.,[18]with some modification; the optical density was determined at 490 nm (the wavelength of maximum absorbance for glucose and starch).

Commercial extract (Pharmaceutical Grade β-glucan)
Imuneks (10 mg/capsule) was purchased, Istanbul Turkey, and 1 mg/ml from Imuneks was prepared by [17]method.

Determination of LD50
Graded doses of β-glucan extract of Saccharomyces cerevisiae in 0.3 ml were administered subcutaneous S.C to each one mouse daily, a series of concentrations of beta glucan local extract were chosen. The ranges of single doses which were used in the determination of LD50 of glucan extract was (200, 400, 600, 800, 1000 µg/ml) .The mortality was recorded after 24hs. Then the LD50 was determined according to the formula employed by Dixon [19].

Transplantation of tumor cells in mice
Preparation of cells for subcutaneous injection in mice:
According to the (15, 20) the following protocol was followed to perform the transplantation process, which carried under highly sterile conditions.
a) The tumor mass region was well disinfected with 70% ethyl alcohol.
b) Ten ml disposable syringes (18 Gage) was used to aspirate the contents of tumor

mass tissue then withdrawn into sterile flask and suspended into 50 ml of sterile PBS.
c) The solid contents were allowed to settle down while the supernatant discarded.
d) The sediments washed 2-3 times with sterile PBS. Generally, then withdrawn content from tumor mass of single mouse was adequate for transplantation of an average 20-25 mouse.
e) The cells suspension was homogenized via vigorous pipetting.
f) One ml of tumor cell suspension was transplanted to the adult female albino mice BALB/C (6 weeks old) through insertion of a needle (gauge no.18) subcutaneously from thigh region toward the shoulder region where the injection was performed.

Treatment of tumor by using soluble β-glucan extract:

Once tumor was reached the suitable volume at least 6 - 9 mm in dimension (20,21). The tumour dimensions were measured with vernia in millimetre (mm) ,mice were randomized arrange into 6 groups :
1 - Six treatment groups (each contains of 10 adult female albino BALB/C mice) were divided as follow:

A – Four experimental groups were injected SC with (1mg/ml) of soluble β-glucan extract of S. cerevisiae ; two groups of mice was administrated with soluble β-glucan before tumor transplantation was considered as (G1) ,whereas the other two groups were daily injected SC of soluble β-glucan after 10 days from tumor transplantation and continued for thirty days (considered as G2).

B - Other two groups (G3) were daily injected SC of (1mg/ml) soluble β-glucan after 24h from tumor transplantation and continued for three weeks.
2- Control groups (G4) were SC tumor transplantation; and SC injected with PBS only.

Tumor measurement: According to (20, 21)

1 -Relative Tumour Volume (RTV) % (mm3):

$$R.T.V.(\text{day x}) = \frac{\text{tumor volume}(\text{day x})}{\text{tumor volume}(\text{day 0})} x100$$

$$\text{Tumor volume (mm3)} = \frac{axb^2}{2}$$

a= length of tumor mass (mm)
b= width of tumor mass (mm)

2- **Tumor growth inhibition (TGI %)** :

$$GI\% = \frac{\text{tumor volu me of untreated group - tumor vol ume of treated group}}{\text{tumor volu me of untreated group}} x100$$

RESULTS

β-glucan extract

The dried particulate of local of Saccharomyces cerevisiae that gave a bright yellow product; and became powder upon drying. Samples containing carbohydrate developed a red-orange colour rather than the amber colour typical of the phenol-sulphuric acid assay. Intensity of the red colour increased with increasing the concentration and compared with commercial extract. The percentage of crude local extract of beta glucan was 12.3%.

Determination of LD50:

The median lethal dose (LD50) of Beta glucan local extract of Saccharomyces cerevisiae through S.C administration in female mice, showed no LD50 to all concentration .The mice showed normal respiration, eating, and behavior , there wasn't signs observed later.

Treatment of tumour by using β-glucan local and commercial extracts (in vivo study)

1 -Relative Tumour Volume (RTV) %:

After the tumour reached to suitable volume and became palpable (7-9) days after the transplantation of tumour cells AMN3; treatment with the soluble β-glucan was given subcutaneous . In (Figure 1); the results showed that the

RTV of the control group at third day was (179) mm3 and at the twenty one day was (389) mm3 as compared with the groups (G3) that treated with local and commercial extracts (108, 114) mm3 and (188, 212) mm3 respectively. While in (Figure 2) the RTV for the control group was (189) mm3at the third day and was (478)mm3 at the thirty day as compared with the groups(G2) that treated with local and commercial extracts (177,181) mm3 and (216 , 241) mm3 respectively . There was a significant differences (P<0.05) between the treated and control groups. A relative tumour volume was not denoted in mice groups G1 (that treated before tumour cell transplantation) .

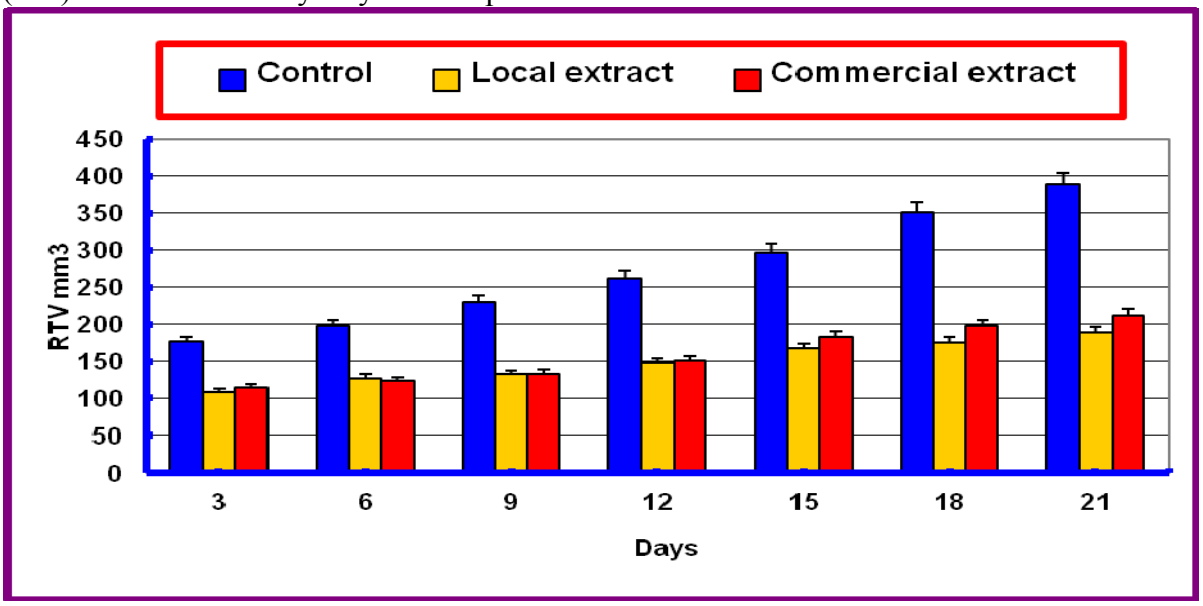

Figure 1. Mean values of relative tumour volume percentage (RTV) % (mm3) of animal groups (G3), that treated within tumour implantation (local and commercial extracts) β-glucan of S. cerevisiae with control group for three weeks. Significant differences (P ≤ 0.05) between treated groups and control group. Significant differences (P ≤ 0.05) between periods.

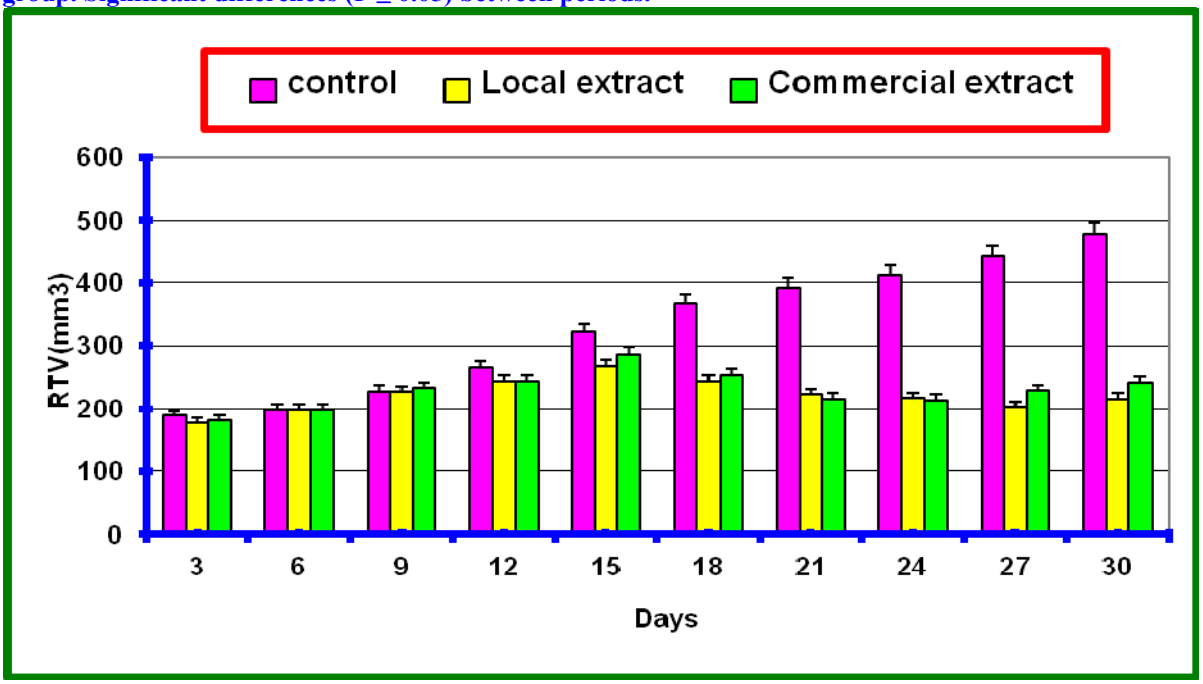

Figure 2. Mean values of relative tumour volume percentage (RTV) % (mm3) of animal groups that treated with (local and commercial extracts) β-glucan of S. cerevisiae after 10 days of tumor cell implantation (G2) with control group for one month. Significant differences(P ≤ 0.05) between treated groups and the control group. Significant differences (P ≤ 0.05) between periods.

2- Tumour Growth Inhibition (TGI) %

The percentage of TGI in different mice groups; showed that the best inhibitory effect on TGI was obtained from the groups (G2) that treated with local extract (54.4%) at day thirty (Table 2), while groups (G3) treated with extract showed the best inhibitory effect (51.67%) at twenty one days (Table 1).

The period of three weeks in (Table 1) refers that all groups have significant differences($P<0.05$) with inhibition percentage (38.6,42.6,50.14,and 51.67)% for local extract,(35.22, 42.17, 43.5 and 45.5)% for commercial extract , so the increase in the tumour growth inhibition depends on the time, except in commercial extract showed decrease in TGI (38.3%) at fifteenth day, also in the local extract showed decrease in TGI (35.8%) at the sixth day. (Table 2) reveals tumour growth inhibition of local and commercial extracts for one month period; the groups had significant inhibitory effect ($P<0.05$); except at the sixth day there was no inhibitory effect and also at the ninth day (0.5, 2.2) % for local and commercial extracts respectively.

Table 1. Mean values of tumour growth inhibition percentage (TGI) % of treated animal groups (G3) that treated with (Local and commercial extracts) β-glucan of S.cerevisiae for three weeks

Types of therapy	Tumour Growth Inhibition (TGI) % Periods of measurement (TGI)% Mean ± SE						
	3 days	6 days	9 days	12 days	15 days	18 days	21 days
Local Extract (β-glucan)	38.63±1.22 a	35.85 ±1.22 b	42.60 ±0.90 a	43.29 ±0.28 a	43.77 ±0.81 a	50.14 ±2.04 a	51.67 ±1.63 a
Commercial Extract (β-glucan)	35.22 ±1.22 b	37.87 ±1.22 a	42.17 ±0.81 a	42.14 ±0.87 a	38.38 ±1.22 b	43.58 ±1.22 B	45.50 ±1.22 b

Small different letter denoted significant between treated groups at level $P \leq 0.05$

Table 2. Mean values of tumour growth inhibition percentage (TGI) % of treated animal groups (G2) that treated with (Local and commercial extracts) β-glucan of S. cerevisiae for one month.

Types of treatment	Tumour Growth Inhibition (TGI) % Periods of measurement (TGI)% Mean ± SE									
	3 days	6 day	9 days	12 days	15 days	18 days	21 days	24 days	27 days	30 days
Local extract (β-glucan)	6.3 ±0.12 a	0 a	0.5 ±0.0 2 b	8.27 ±0.11 a	17.1 ±1.22 a	33.78 ±1.43 a	42.96 ±1.22 b	47.33 ±0.81 A	50 ±2.04 a	54.4 ±1.63 a
Commercial extract (β-glucan)	4.23 ±0.01 b	0 a	2.2 ±0.1 2 a	8.64 ±0.81 a	11.2 ±0.40 b	31.06 ±1.22 b	44.75 ±1.63 a	48.1 ±1.63 a	48.2 ±1.63 b	49.58 ±1.63 b

Small different letter denoted significant between treated groups at level $P \leq 0.05$

DISCUSSION

The extract of *S. cerevisiae* yield crude extract (12.3%) which was greater than (8.7%) of beta glucan extracts of *Poria cocos* [23,24]. differences between percentage of extracts may be due to somelost during the processing and the type of preparation to each one. The extract showed fine bright yellow powder and sticky [16,17] ,but was dark brown sticky for hot aqueous extract of *Poria cocos* .

Median lethal dose LD50 test considered one indicator of the toxicity of a substance that kills half of the laboratory animals[19] . Local extract β-glucan have no side

effects, and there are no LD50 in mice after subcutaneous administration . Such products have received the generally regarded as safe rating by the food and drug (FDA) [24].

The transplantation of AMN-3 was successfully appearing in inoculated healthy female mice during 2-3 weeks. The mass of tumour then was ready for treatment when it reached at least 7-9 millimetre (mm) in any dimension. The control group showed advanced in growth of tumour and the mass was enlarged which soft in consistency and indurate and sometime became ulcerated. Whereas in treated mice the tumour mass was smaller in size and dry with little ulceration in some cases. Large area of necrosis was occurred in the skin of control group of mice that noticed around the area of tumour mass, and this may be due to rapid division of cancer cells that surpass the ability of new blood vessels to supply it with adequate amount of nutrient and oxygen [26.]

Failure of transplanted cancer cells in animals group that treated before tumor cell implantation (G1) attribute to non-specific defence mechanisms, β-glucan have attracted attention in the fields of biochemistry and pharmacology for their immunopotentiation and anti-tumor effects [26-28]. The anti-tumor activities of glucan are mostly resulted from their immunopotentiation effects [29].Its can stimulate immune cells such as granulocytes, monocytes, macrophages and nature killer cells (NK)to trigger the secretion of cytokines that will stimulate the immune system [30] , it can interact with the receptors of immune cells to trigger immunological responses including anti-tumor activity [31,32],so the NK and lymphokine-activated killer cells (LAK) inhibit the tumour growth of the animals, which have the same activity in human tumours. The non- specific defense mechanisms may have an important effect in the failure of transplanted cancer cells [33].

The transplanted groups of mice treated with local and commercial β-glucan extract was reported significant effect in all the periods of the experiment (Figures1&2) for relative tumour volume (RTV), and (Tables 1&2) of TGI for the periods of (three weeks & one month) respectively.

Tumour growth inhibition of treated groups that treated after tumor implantation (G2) with β-glucan extracts of Saccharomyces cereivisae ,showed significant inhibition ($P<0.01$) of tumour growth in all groups of local and commercial extracts at all periods. These extract have shown an effect by increasing the life span of animals [34,35] Ma et.al. , [8] proved that the Auricularia auricular of fruit bodies caused a significance inhibitory effect on the experimentally induce adinocarcinoma in mice.

Analysis of the response of human and mouse leukocytes to β-glucan has shown that the complement receptor CR3 is primarily responsible for the high affinity binding of soluble and particulate β-glucans and the cytotoxic and phagocytic responses mediated by β-glucan stimulated macrophages, neutrophils and NK cells [36] , in addition to CR3, dectin-1 a specific β-glucan receptor [29]. Dectin-1 is expressed on macrophages, monocytes, granulocytes, dendritic cells and also on B cells and a subpopulation of T cells [37]. On macrophages,dectin-1 is the dominant receptor mediating the phagocytosis of glucan. Although macrophages and granulocytes may also use dectin-1 to capture glucan [33], only CR3 with bound β-1,3-glucan triggers cytotoxic degranulation in response to inactivated complement 3b(iC3b) fragment coated tumors ,previous reports also suggest that β-glucan can promote T cell-specific response , and shown that there was a marked deregulation in the balance between Th1 and Th2 immune response in the course of cancer, being reported dominant Th2-type responses as a consequence of the progressive loss of Th1-type responses in

tumor-bearing animals [38]. In conclusion the soluble β-glucan of S. cerevisiae (local & commercial extracts) had a great potent cytotoxic effect against tumor cells line AMN-3 in mice

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پوختە

خواندنا کارتێکرنا (البیتا گلوکان) یا رونکری بو هەڤیرتشا نانی Sacharomyces Cerevisiae شینبوونا شانێن شیرپەنجی (AMN3) دناڤ لەشێن ساخدا

پێشەکی و ئارمانج: ئەڤ خواندنە هاتە ئەنجامدان ب مەرەما ل دویف چوونا رووڵ ژەهراوی یێ (البیتا گلوکان) ئەوا هاتیە وەرگرتن ژ هەڤیرتشێ نانی S. Cerevisiae ل سەر شینبوونا شانێن شیرپەنجی (AMN3) دناڤ لەشێن ساخدا.

ڕێکێن ئەکولینێ: دەستنیشانکرنا دوسا نیف کوژەك (LD50) ل سەر مشکێن ساخ پشتی شرینقەدانا وان ب (البیتا گلوکان) ئەوا هاتیە وەرگرتن ژ هەڤیرتشێ نانی S. Cerevisiae دبن چەرمی دا و ئەنجا دیارکر چ رووڵەکێ ژەهراوی نەبو بو ڤی هەڤیرتشی ل ڤان ڕێژا (200–400–600–800–1000) میکروگرام / ملیلیتر. شانێن شێرپەنجی یێن لبنی (AMM3) هاتنە ب کارئینان بو زانینا کارتێکرنا (البیتا گلوکان) ل سەر ڕێژا شێرپەنجا (RTV).

ئەنجام: دیار بو کو رووڵ کارتێکرنا (البیتا گلوکان) ئەوا هاتیە دروستکرن ب شێوەکێ خومالی ل سەر کێمکرنا ڕێژا شێرپەنجێ ب هێزتر بوو بەرامبەر(البیتا گلوکان) یا (تجاری) ل سەر گروپێن هاتینە شرینقەکرن پشتی بورینا 24 سعەت و 10 روژان ب شانێن شێرپەنجی (AMN3) . سەبارەت گروپێن هاتینە شرینقەدان بەری 10 روژان ب هەردوو (البیتا گلوکان) یێن خومالی و تجاری: هیچ نیشانێن شێرپەنجێ د مشکێن هاتینە شرینقەکرن ب شانێن شێرپەنجیێن لبنی (AMN3) دیار نەبوون چەنکی ئەڤی پروتوکولەکێ باشتر بو چارەسەریا شێرپەنجێ هاتە بکارئینان بو ڤی گروپی بەراوردکرن دگەل هندەك گروپێن دی.هەروەسا ئەنجامێن بەرچاڤ (P<0.05) هاتنە دیتن د ڕێژا کێمکرنا بەلافبوونا پیڤەرێ شێرپەنجێ TGI (% 51.67,38.6,42.6,50.1) دەربارەی (البیتا گلوکان) یێ خومالی و (% 45.5,43.5,42.17,35.22) یێ تجاری بو گروپێن هاتینە چارەسەرکرن پشتی بورینا 24 سعەتا ژ چاندنا شانێن شیرپەنجی.

دەرئەنجام: (البیتا گلوکان) یێ رونکری کارتێکرنەکا باش هەبو ل سەر کێمکرنا ڕێژا زێدەبونا شانێن شیرپەنجی (AMN3) ل دەف مشکا.

الخلاصة

دراسة تأثير مستخلص البيتا كلوكان الذائب لخميرة الخبز (Saccharomyces cerevisiae) على نمو الخلايا السرطانية AMN3 داخل الجسم الحي

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.

الخلفية والأهداف: صممت هذه الدراسة للتحري عن التأثير السمي للبيتا كلوكان (المستخلص والكلوكان التجاري) المستخلص من خميرة الخبز S. cerevisiae للخطوط الخلوية السرطانية AMN3 داخل الجسم الحي.

المواد و الطرق: تم تحديد الجرعة النصفية المميتة LD50 للفئران السليمة بعد حقنها بمستخلص البيتا كلوكان تحت الجلد المستخلص من خميرة الخبز S. cerevisiae ولم تظهر النتائج اي سمية للمستخلص عند التراكيز (200و400و600و800و1000) ميكروغرام/مليليتر المستخدمة في التجربة .استخدمت الخلايا السرطانية اللبنية AMN3 لمعرفة تأثير البيتا كلوكان على معدل الورم النسبي RTV للورم السرطاني

النتائج: أظهر مستخلص البيتا كلوكان المحضر محليا تأثيرات تثبيطية اعلى في معدل الورم النسبي للورم السرطاني مقارنتا مع البيتا الكلوكان التجاري للمجاميع المحقونة بعد 24 ساعة و 10 ايام بالخلايا السرطانية AMN3 اما المجاميع المعالجة قبل 10 ايام بالمستخلصين لم تظهر اي ورم سرطاني في الفئران المحقونة بالخلايا السرطانية اللبنية خلال فترة التجربة اذ اعطت هذه المجموعة بروتوكول افضل لعلاج السرطان مقارنتا مع المجاميع الاخرى. . كما بينت النتائج تأثيرا في معدل تثبيط النمو السرطاني TGI (38.6,42.6,50.14 و 51.67٪) بالنسبة للمستخلص المحلي و(35.22 42.17 و 43.5 45.5٪) للكلوكان التجاري للمجاميع المعالجة بعد 24 ساعة من غرس الخلايا السرطانية والتي اعطت فرقا معنويا ($P<0.05$) اذ اعتمدت الزيادة التدريجية في معدل نسبة اثباط النمو السرطاني للمستخلص المحلي و التجاري على فترة الحقن المستخدمة في التجربة ما عدا في مجموعة الكلوكان التجاري أظهر انخفاض في معدل تثبيط النمو السرطاني بنسبة (38.3%) في اليوم الخامس عشر، و (35.8٪) بالنسبة للمستخلص المحلي عند اليوم السادس.

الاستنتاجات: مستخلص البيتا كلوكان الذائب كان له تأثير تثبيطي فعّال على نمو الخلايا السرطانية AMN-3 في الفئران.

ANTERIOR CERVICAL DISCECTOMY AND FUSION: A PRELIMINARY REPORT ABOUT DUHOK EXPERIENCE

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ABSTRACT

Background: Anterior cervical approach has, for many decades, been a well established and standard access to degenerative, traumatic and neoplastic cervical spine lesions achieving a substantial benefit to the patient population.

Design and Setting : This is a retrospective study encompassing twenty adult patients, who had been operated upon, "anterior cervical discectomy and fusion" at the Neurosurgical Department, the Accident and Emergency Teaching Hospital in Duhok City between April 2010 – April 2012, by the neurosurgical staff as a team approach. The follow-up period ranged from 6 months to 2 years.

Aim of study

An initial and preliminary report spreading the Duhok experience in this respect of neurosurgical practice.

Patients and methods: There were twenty adult patients, 12 males and 8 females, ages ranged from 18 – 60 years, mean 39 years and 4 months. Diagnosis was achieved via clinical history and examination, plain cervical spine X-ray radiography, computed tomography (CT) and magnetic resonance imaging (MRI); other ancillary clinical investigations were done like neurophysiologic tests. The surgical procedure was the standard anterior cervical discectomy with fusion. Polyetheretherketone (PEEK) cages were used utilizing Ulrich Co. instrumentation system aided by image intensifier localization and the use of operating microscope (Pantera of Zeiss Co.). All patients had post-operative imaging checking.

Results: There were 16 patients with symptomatic degenerative and 4 patients with traumatic cervical spine lesions. Post-operatively, all patients experienced various degrees of immediate relief of symptoms with marked improvement of radicular and neck pain in 93.75% of the patients with degenerative spine disease. However, those with traumatic spinal cord injury had slow neurological recovery. Transient complications were in form of hoarseness, swallowing difficulty and C5 pain were mild and seen in 20% of cases. Permanent and surgical and cage related complications were not encountered.

Discussion: The indications for the surgical intervention were symptomatic degenerative cervical disc disease and spondylotic myelopathy in 16 cases (Fig. 1 and 2) and cervical spine trauma (4 cases). The lower cervical spine segment involvement (C5-6-7 levels) was noticed in 13 (81.25%) out of 16 patients with degenerative spine disease. Overall, single level surgery was done in 6 (30%) patients while the rest 14 (70%) patients underwent two levels surgery. The implants included either single-level interbody cage insertion or two-level-cage insertion with, additional, plate and screws fixation.

Conclusions: It is concluded that the team neurosurgical approach to the anterior cervical pathology, in the availability of standard facilities, is very fruitful and can achieve an excellent clinical results to the patients.

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Key words: Degenerative Cervical Lesion, Traumatic Cervical Lesion, Anterior Cervical Discectomy and Fusion, Polyetheretherketone.

Anterior Cervical Discectomy and Fusion (ACDF) remains the gold standard for the surgical management of cervical spondylotic radiculopathy and myelopathy. The principal aim of the operation is to remove disc material and osteophytes causing neural compression.

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The operation can be performed for multilevel (1-3 levels) compression, gives direct access to the offending pathology, resulting in a favorable outcome in 80–90% of patients [1, 2].

Objective clinical signs of myelopathy and certain radiculopathies associated with spinal cord and nerve root compression from disc herniations and/or osteophytes seen on MRI scans, CT scans, or CT myelography are indications for an anterior approach.

Interbody fusion is achieved by insertion a bone graft harvested from the patient or an artificial cage into the disc space. A number of synthetic bone grafts and cages have been used as a substitute for autologous bone graft. The cage has been shown to reduce the complication rate by 22% in comparison with autogenous iliac crest graft fusion[3]. Also bone graft donor site pain remains a drawback of ACDF which is reported in up to 31% of patients.[1] Cages also restore disc height, restore cervical lordosis, avoid donor site pain and reduce operative time. Additional advantages of fusion are stability of the cervical spine, preservation of the physiological lordosis, reduce neck pain and prevent post operative foraminal narrowing [4, 5].

Many different cage designs (e.g. cylindrical, mesh, ring or box shaped) and materials (e.g. titanium, carbon, polyetheretherketone, hydroxyapatite coated) have been introduced [4, 6] . Each one has its own advantages and drawbacks.

METHODS

This study includes 20 patients with mean age of 39 years, ranging from 18 to 60 years. There were 12 males and 8 females. All were operated for anterior cervical microdiscectomy and fusion at the Neurosurgical Department, Accident and Emergency Teaching Hospital in Duhok city between April 2010 and April 2012. Follow-up period ranged from 6 months and 2 years.

Sixteen patients were treated for degenerative cervical disc disorders, the rest for cervical spinal trauma. Among 16 patients with cervical disc degenerative disorders, presenting symptoms included radiculopathy in 12 (75%), myelopathic symptoms in 2 patients (12.5%) and myeloradiculopathy in another 2 (12.5%) patients. The preoperative diagnosis was performed by MRI in all 16 cases. Additional cervical spinal CT scan examination was done for spinal trauma cases.

In all patients with radicular and neck pain, the discectomy and fusion were proposed after three months of unsuccessful conservative measures.

Informed Consent was taken from all patients. The surgical procedure was the standard anterior cervical discectomy with fusion, done under microscopic magnification using Pantera Zeiss operating microscope aided by image intensifier localization of the level. The fusion was performed using the PEEK cages utilizing the Ulrich fixation system. All patients received perioperative 1 gm intravenous ceftriaxone antibiotic prophylaxis.

Cervical collar application was used for 2 weeks to avoid excessive cervical motion.

Clinical and radiographic controls (including standard and flexion-extension radiographs) were conducted at periodic intervals after surgery (1st post-operative day, 1 month, 3 months, 6 months and 12 months after surgery).

At each visit the level of patients' satisfaction was assessed by questionnaires regarding the postoperative residual pain using the visual analogue scale from 0 to 10, assessment of the motor function if weakness was present pre-operatively, the need for postoperative analgesics and the time period taken for the patient to join his work.

The outcomes were considered as "very good", "good" or "fair" depending on

complete resolution of the pre-operative complaints, if there is transient post-operative complications and if there is non-consistent improvement of pain and the pre-operative neurological changes respectively.

RESULTS

The indications for surgical intervention in our patients were symptomatic degenerative diseases of the cervical spine and unstable cervical spine injury.

Cervical disc disease was seen in 12 cases, spondylotic myelopathy in 4 cases and cervical spine trauma in 4 cases.

Clinically, all 12 patients with degenerative disc lesions were presented with radicular pain with paresthesia in the corresponding nerve root territory. Two of them had associated early motor weakness. In contrast, all patients with cervical spondylotic myelopathy were presented with grade 3-4 spastic weakness of both upper and lower limbs. Three patients with spinal trauma were due to road traffic accident. The other one patient had history of fall from height. Two of these patients were presented with features of anterior cord syndrome, whereas the others were presented with neck pain and radiculopathy.

Among trauma cases, 2 patients underwent one level ACDF for C3-C4 and C4-5 respectively. The other 2 patients underwent two levels surgery for C4-5-6 and C6-T1 (with corpectomy to C7).

The rest 16 patients presented with spondylotic cervical degenerative diseases. Of these, 4 patients had single level surgery for C3/4 (1 case), C4/5 (1 case) and C5-6 (2 cases). The rest 12 patients underwent two levels surgery which includes: 1 patient for (C3-4-5), 6 patients for (C4-5-6) and 5 patients for (C5-6-7) levels. The lower cervical spine segment involvement (C5-6-7 levels) was noticed in 13 (81.25%) out of 16 patients. Overall, single level surgery was done in 6 (30%) patients while the rest 14 (70%) patients underwent two levels surgery.

Hospital stay after operation was 3 days, without major complications and with a low requirement of analgesics during the first post-operative days.

Permanent hoarseness or swallowing complaints were never observed. Transient hoarseness was seen in 2 (10%) patients lasted up to 8 weeks. Of these, 1 patient had degenerative spine disease and the other had spinal trauma. Transient swallowing difficulties were observed in 1 (5%) patient. Another 1 patient was complaining of C5 root pain for 2 days post-operatively. The peri-operative mortality was nil (0%).

In degenerative spine disease group, follow-up evaluation of the outcomes at the end of the 6th post-operative month showed that 1 (6.25%) patient with spondylotic myelopathy had fair outcome, 2 (12.5%) patients experienced "good" results and the rest 13 (81.25%) patients had "very good" outcome. Thus sustained improvement of the initial neurological changes with marked improvement of radicular and neck pain was noticed in 93.75% of the patients with degenerative spine disease.

Patients with vertebral and spinal cord injury showed slower neurological recovery. Implant related complications were never observed in our patients. No patient in this study required re-operations whether early or late.

DISCUSSION

The past decade has witnessed significant advances in the surgical treatment of cervical myelopathy and myeloradiculopathy.

There is a large bulk of evidence confirming the safety and efficacy of the ACDF. The procedure has been widely used as an ideal surgical treatment for cervical disc degenerative disorders.[7] It has been demonstrated to be effective in the treatment of cervical disc degenerative

disorders in many reports, and the advantages of this procedure may include direct decompression of spinal cord and nerve roots, immediate stability of involved segments, and restoration of cervical lordosis and intervertebral height [8].

Typical presentation of spondylotic disc lesion or myelopathy was seen in all of our patients with degenerative diseases of the cervical spine. There was high incidence of lower cervical spine involvement in our patients. Similar results had been presented in other studies [9, 10].

It has been stated that the most important outcome predictive factor is the correct determination of operative indications [9] and more reports suggest a significant benefit with surgical intervention in appropriately selected patients [11,12]. Several outcomes studies in the literature demonstrate overall good or excellent results in 70% to 89% after ACDF [13, 14, 15] and significant pain relief in 93% of the patients[11] .In another recent study, 78.9% of patients rated their level of satisfaction as excellent and good in the ACDF [16] .The overall surgical outcome (good and very good outcome) in our patients with degenerative cervical spine diseases indicated marked improvement of radicular and neck pain in addition to sustained neurological improvement of the preoperative changes in 93.75% of patients. This is comparable to the results shown in the literature.

Because the majority of our patients (81.25%) showed a very good response to treatment, it is presumed here that both age and sex has no influence on the outcome. This is in contrast to other reports which considered young age of the patient as a predictor of a satisfactory outcome [9] .The predominance of the lower cervical segment involvement in our patients can also contribute to the marked response noticed in this study. This is in agreement with others who reported that ACDF to the lower cervical spine is considered as predictors of a satisfactory outcome.[9]

Fourteen (70%) patients in this study underwent two levels surgery. Multiple level discectomy and fusion should be considered for nerve root compression at multiple levels with significant axial neck pain or significant narrowing of the neural foramen by osteophyte formation[17]. According to the outcome of our patients, the current study clearly demonstrates the feasibility of the ACDF procedure in addressing the multiple ventral compressive pathologies and effectively dealing with the degeneration process that is at or near the disc spaces.

The traditional autograft from the ileum used to achieve fusion, documenting high fusion rate, was often reported to have donor site complications [18]. To overcome this problem, synthetic interbody cages with different materials and designs have been developed and are very popular in the treatment of cervical disc degenerative disorders. Among these cages, PEEK was considered as an enticing alternative to bone graft[19]. PEEK cages were used in our patients to achieve fusion after discectomy. We found that PEEK cages were simple to use, easy to handle and its central cavity can be packed with bone. More advantages include: having a modulus of elasticity that approaches normal bone, causes minimal inflammatory response, and it is radiolucent, therefore, it is easy to assess the fusion status 19.

Concerning complications, major complications were not noticed in this study while the types of complications seen were only temporary and well described in the literature. One of a recognized complication of ACDF surgery is recurrent laryngeal nerve (RLN) palsy. The RLN is often exposed in the lower part of the wound at the C5/6 level. 20 The reported incidence of symptomatic RLN after ACD varies between 0.07 and 11% [21]. A prospective study utilizing pre- and post-operative indirect laryngoscopy revealed an overall incidence of 24.2% [22] .Interestingly, transient hoarseness was seen in 2 (10%) of our patients only which

lasted for 8 weeks. The rest of noticed complications were mild and not permanent.

CONCLUSIONS

This is a preliminary report about our experience with anterior cervical discectomy and fusion surgery in our locality. It showed that the procedure is safe and effective means of treating both spinal degenerative cervical diseases and traumatic cervical lesions with very good clinical results for both single and multiple levels. All disc patients have very good outcome. Sustained and marked improvement was noticed in 93.75% of the patients. Trauma cases showed slower neurological recovery. Age and sex has no predilection to the satisfactory outcome. These findings are comparable to the literature. The rate of complications was low and all were transient. No major complications were noted and cage related complications were never noticed in this study. Peri-operative mortality was nil (0%) and no patient in this study required early or late re-operations.

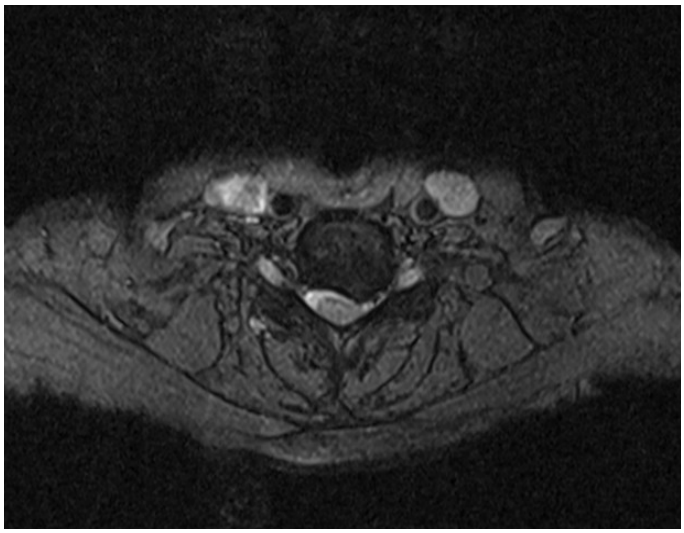

Figure 1. Axial T2 weighted MRI examination at the level of C5-6 showing left sided disc lesion with compression of the nerve root.

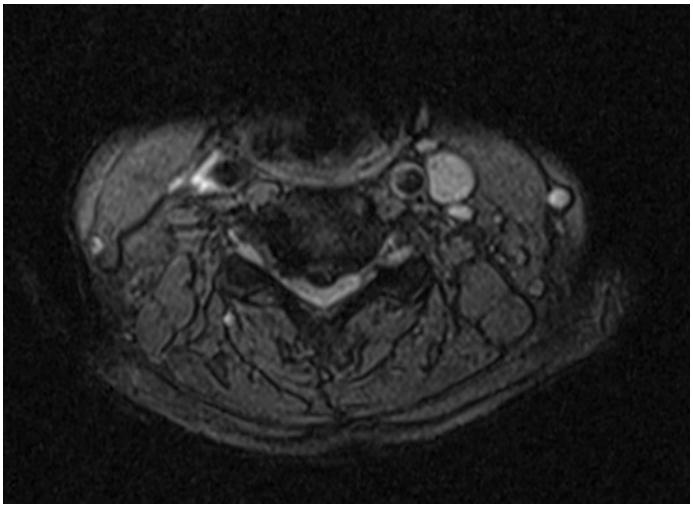

Figure 2 Axial T2 weighted MRI examination at the level of C4-5 showing severe compression of the spinal cord causing advanced spondylotic cervical myelopathy.

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پوختە

راکرنا دیسکێ دناڤبەرا بربریت ستوی دگەل موکمکرنا بربران ژ لایێ پێشیێڤە:

راپورتەکا دەستپێکی ل سەر ئەزمونا دهوکێ

دەستپێك: راکرنا دێسکی د ناڤبەرا (دگەل موکمکرنا) بربرێت ستوی ژ پێشییڤە , ئەڤە ب دەهان سالایە , رێکەکا باوەرپێکری و بنەرەتە ژ بۆ سەرەدەریێ دگەل ئێشێن برپرێن ستوی ئەوێن پەیدابن ب ئەگەرێ تێکچونێ دگەل دەمی , یان ب رویدانان , و یان ب گرێك و پەنجەشێرێ , و شیایە مفایەکی زۆر بگەهینیتە نەخوشان.

ئامادەکاری و دیزاین:ئەڤ ڤەکولینە (20) بیست نەخوشان ب خوڤە دگریت کو بەری نهو نیشتەرگەری بۆ وان هاتیە ئەنجامدان (رێترۆسپێکتیڤ) , (12) نێر و (8) مێ , ژیێ وان د ناڤبەرا (18 – 60) سالێدا بوون (رێژا ناڤین 39 سال و 4 هەیڤ) ل پشکا نیشتەرگەریا کوما دەمارا \ نەخوشخانا تەنگاڤیا فێرکاری \ دهوك , د ناڤبەرا (نیسانا 2010 – نیسانا 2012) ژ لایێ تیمێ نیشتەرگەریا کوما دەماراڤە . ماوێ دویڤچوونا نەخوشان (6 هەیڤ – 2 سال) بوو.

ئارمانج:راپورتەکا دەستپێکی ل سەر بەلاڤکرنا ئەزمون و شارەزاییا دهوکێ د بواری نیشتەرگەریا کۆما دەمارادا.

نەخوش و رێکێن بجهئینانێ:

هەژمارا نەخوشان (20) بیست بوون (12 نێر و 8 مێ) ژیێ وان د ناڤبەرا (18 – 60) سالییێدابوو (رێژا ناڤین 39 سال و 4 هەیڤ) . دەستنیشانکرنا نەخوشیێ هاتیە کرن ب رێکێن پشکنینا نەخوشی ژ لایێ نوژداریڤەو X-ray , CT scan ,MRI ب کارئینانا ئامیرێت تیشکێ

هەروەسا دەستنیشانکرنا کارکرنا دەمارا (نیوروفیزیولوجی) , نیشتەرگەریا راکرنا دیسکی دناڤبەرا برپرێن ستوی دگەل موکمکرنێ ژ لایێ پێشییڤە هاتیە ئەنجامدان دگەل ب کارئینانا دیسکەکێ چێکری ژ کەرستێ (پییك) ب رێیا ئامیرێت (اولریخ) و بکارئینانا ئامیرێ وێنێن تیشکی و میکروسکوپەکا پێشکەفتی ژ جورێ (پانتێرا زایس) د ناڤ نیشتەرگەریێدا. پاش نیشتەرگەریێ وێنێن تیشکی (ژ بۆ پشتراستبوونێ) بۆ هەمی نەخوشان هاتینە گرتن.

ئەنجام:شازدە کەس (16) ژ ڤان نەخوشان توشی تێکچوونا برپرا ببوون دگەل دەمی , و (4) ب ئەگەرێ رویدانا . پشتی نیشتەرگەریێ هەموو نەخوشان مفایەکی بەرچاڤ دیتن ب تایبەتی ئێشانا باتکێ و ملی (د 93.75٪) دا ژ نەخوشێن توشی تێکچوونێ بووین دگەل دەمی , بەلێ نەخوشێن توشی رویدانا بووین و دەمارا پشتێ هەرشی باشبوونەکا هێدی ب سەردا دهات.

ژ بلی ئاریشێن کێم و بەروەخت ژ ئەگەرێ نیشتەرگەریێ وەکو (بۆرپۆنا دەنگی , و قورچا ب زەحمەت , و ئێشانا ملی) کو 20٪ ژ نەخوشان توشبوون , چ ئاریشێن دی رویندان.

گەنگەشە:ئەگەرێن نیشتەرگەریێ فشاربوو ل سەر دەماریت ستوی ب ئەگەرێ تێکچوون و ژ جهچوونا برپران دگەل دەمی (دیجینیَرێتیڤ) ل لایێ (16) نەخوشان , و ب ئەگەرێ رویدانا (4) کەس بوون. ئاستێن نیشتەرگەری بو هاتینە ئەنجامدان ب ڤی رەنگیبوون:(1 کەس) C6/C7/C8 , (7 کەس) C6/C7 , (10 کەس) C5/C6 , (7 کەس) C4/C5 , (2 کەس)C3/C4

نیشتەرگەری ژ بو (4) نەخوشان ل ئێك جهـ (1 ئاست) هاتیە کرن, و (16) نەخوشێن دی ل پتر ژ ئێك جهـ هاتیە ئەنجامدان و جارجار ب پلێت و بورغیا هاتیە موکمکرن.

رامان و بەرهەمهاتن:بو مە دیار بوو کو نیشتەرگەریا کوما دەمارا ژ لایێ پێشییڤە (ژ لایێ تیمێ نوژداریڤە) ژ بو ئاریشێن پربرا ستوی , دگەل بدەستکەفتنا ئامیرە و شیانێت پێتڤی , زۆر یا ب مفایە بو نەخوشییێن برپرا ستوی.

الخلاصة

استئصال القرص العنقي الأمامي والاندماج: تقرير أولي حول تجربة دهوك

الخلفية:ان النهج الجراحي الامامي للفقرات العنقية, ولعقود عديدة, اعتبر من الطرق الجراحية الراسخة لعلاج آفات واصابات الفقرات العنقية والذي ادى الى تحقيق فائدة كبيرة للمرضى.

التصميم والاعداد: هذه دراسة استعادية تشمل 20 مريضا ، اجريت لهم عمليات استئصال القرص الفقري العنقي مع الاندماج في شعبة جراحة الأعصاب، مستشفى الحوادث والطوارئ التعليمي في مدينة دهوك بين أبريل 2010 – أبريل 2012، من قبل جراحي الأعصاب باعتباره نهج الفريق. تراوحت فترة المتابعة من 6 أشهر إلى 2 سنة.

الهدف من الدراسة: قرير أولي وتمهيدي يبين تجربة دهوك في هذا الصدد من ممارسة جراحة الأعصاب.

المرضى والطرق: كان هناك 20 مريضا ، 12 من الذكور و 8 من الإناث، تراوحت أعمارهم من 18 حتى 60 سنة، بمعدل 39 سنة و 4 أشهر. وقد تحقق التشخيص عن طريق التاريخ الطبي والفحص بالأشعة السينية، التصوير المقطعي (CT) والتصوير بالرنين المغناطيسي (MRI)، وأجريت التحقيقات السريرية الأخرى المساعدة مثل الاختبارات الفيزيولوجية العصبية. وكانت العملية الجراحية على مستوى استئصال القرص الفقري مع الاندماج من الناحية الامامية. واستخدمت أقفاص Polyetheretherketone (PEEK) لغرض الاندماج باستخدام نظام تثبيت شركة أولريخ وبمساعدة جهاز الاشعة المكثفة للصورة، واستخدام المجهر الجراحي (بانترا من شركة زييس). وتم فحص جميع المرضى شعاعيا بعد الجراحة .

النتائج: كان هناك 16 من المرضى الذين يعانون من أعراض مرض آفات الفقرات التنكسية و 4 من رضح الفقرات العنقية. بعد العملية، اكتسب جميع المرضى تحسنا بدرجات متفاوتة من الاعراض الملازمة مع تحسن ملحوظ في آلام جذورالاعصاب وآلام الرقبة في 75،93٪ من المرضى الذين كانوا يعانون من أمراض العمود الفقري التنكسية. اما مرضى الرضح الفقري العنقي واصابات النخاع الشوكي العصبية فان تحسنهم السريري كان بطيئا. وكانت المضاعفات العابرة مثل بحة الصوت وصعوبة والبلع وألم الجذرالعصبي الخامس C5 خفيفة ومؤقتة، وشوهدت في 20٪ من الحالات. لم تشاهد مضاعفات ذات صلة دائمة بالجراحة او تلك المتعلقة بالقفص (القرص الصناعي).

المناقشة: ملت دواعي التدخل الجراحي أعراض الأمراض التنكسية للقرص الفقري العنقي واعتلال النخاع الشوكي (16 حالة) ورضح الفقرات العنقية (4 حالات). وقد لوحظ اصابة المستوى الفقري السفلي للعنق (بين مستوى الفقرات العنقية C5-6-7) في 13 (81.25٪) من أصل 16 مريضا يعانون من أمراض العمود الفقري التنكسية. وبشكل عام، فقد تم اجراء جراحة لمستوى منفرد من العنق في 6 (30٪) من المرضى والباقي 14 (70٪) من المرضى خضعوا لعملية جراحية لمستويين. وشملت الجراحة لدى مرضى الرضح تثبيت القفص بين الجسمين مع صفيحة وتثبيت البراغي.

الاستنتاجات: خلص هذا البحث إلى أن نهج فريق جراحة الأعصاب في علاج امراض الفقرات العنقية ، في توافر المرافق القياسية، مثمرة للغاية، ويمكن تحقيق نتائج سريرية ممتازة للمرضى.

NURSE'S KNOWLEDGE ABOUT EFFECTIVENESS OF NATURAL FAMILY PLANNING METHODS

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ABSTRACT

Background: Natural family planning methods are considered easy methods and have no side effects when used to prevent unintended pregnancy, so nurses have an important role in providing counseling and health education for women in fertile age.
Objective: To assess nurses knowledge about effectiveness of natural family planning methods to avoid unwanted pregnancy.
Methods: A descriptive study was conducted on probability sample (systematic random) of (100) nurse and midwife who work in obstetric and gynecological departments from six hospitals at Baghdad City which include family planning clinic which include: AL-Karkh maternity Hospital, Al-Elwia Maternity Teaching Hospital, Fatima Al-Zahra'a Maternity and Pediatric Teaching Hospital, AL-Yarmouk Teaching Hospital, AL-Kademya Teaching Hospital and AL-Nuaaman Teaching Hospital. Study implemented for the period of February 24th 2012 to April 25th 2012. A questionnaire was used as a tool of data collection to fulfill with objective of the study and consisted of four parts, including demographic, reproductive characteristics, personal experience in using method or in attending continue education training of knowledge and effectiveness for each methods of natural family planning. A pilot study was carried out to test the reliability of the questionnaire and content validity was carried out through the 17 experts. Descriptive statistical analyses were used to analyze the data.
Results: The results of the study revealed that most mothers their average age (39) years, (39%) of study sample was graduated from secondary school of nursing, (53%) of study sample work at obstetric and gynecological ward, (84%) of study sample were married and the highest percentage (44%) their sources of knowledge about natural family planning were from Friends and family. Most of the study sample had unaccepted knowledge about effectiveness of natural family planning methods, one reason for this is that little information on natural family planning is provided in nursing curriculum and also because of sources of nurses about natural family planning was not equability enough to based on it.
Conclusion: Nurses had inadequate knowledge regarding the effectiveness of different types of natural family planning because of not have awareness about these methods and not have equability source of knowledge about natural family planning.

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Key words: Knowledge, Effectiveness, Natural Family Planning Methods.

Natural family planning (NFP) refers to a variety of methods used to prevent or plan pregnancy, based on identifying a woman's fertile days [1]. Fertility awareness methods (FAMs) are appropriate for women with regular menstrual cycles. They involve monitoring the cycle and having intercourse only during infertile phases or using another method, e.g. condoms, during fertile phases. A woman cannot identify the exact day of ovulation using FAM methods; rather she identifies when the fertile phase of her cycle begins and ends. A woman's fertile phase may begin 3-6 days before ovulation (because sperm can live in cervical mucus for 3-6 days); a woman's fertile phase ends 24 hours after ovulation [2]. Several names have been used to describe this approach to contraception—rhythm, natural family planning, periodic abstinence and fertility awareness methods. NFP is actually more effective if applied with discipline than most forms of artificial birth control. This may come as a surprise to some physicians who don't pay any attention to NFP [3].

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Fertility awareness or natural family planning has been used successfully since the 1930's to predict woman's fertile days. These natural methods are based on the fact that fertilization is most likely to occur around the time of ovulation. Intercourse is avoided during those times when a woman is fertile, thus preventing conception [3]. Natural family planning methods are methods which use the body's natural physiological changes and symptoms to identify the fertile and infertile phases of the menstrual cycle [4]. There are different methods of natural family planning which include: Standard Days Method (SDM), Calendar-rhythm method, Two-Day method (TDM), Symptothermal Method (STM), Ovulation method, Basal body temperature method (BBT), and Lactational amenorrhea method (LAM) [3,5]. The effectiveness and advantages of NFP attend to needs of varied populations with varied religious and ethical beliefs. They also provide an alternative to women who wish to use natural methods for medical or personal reasons [1]. The results of several studies have found the natural methods to be comparable in effectiveness to contraceptive pills and the intrauterine device, although proper teaching and motivation of couples is required for their successful application. Knowledge of these methods is invaluable to Nurses [6]. The effectiveness of NFP will have: 91- 99 % with perfect use; 75% with typical use. Typical use is defined as a term when couples follow the direction of the process but there may be inconsistencies in charting. The term perfect use defines couples who comply with direction and consistently, couples who practice perfect use of NFP will have 98% percent success of preventing pregnancy [2]. The objectives of present study were to assess nurses knowledge about effectiveness of natural family planning methods to avoid unwanted pregnancy.

METHODS

Descriptive study was carried out upon nurses who work at Baghdad city hospitals in gynecological and obstetric departments. Study implemented for the period of February 24th 2012 to April 3rd 2012. Data collection will be gathered by questionnaire format and Interview with nurses at Baghdad city hospital. Probability sampling approach; (systemic random sampling) was utilized through following steps:

1. Researcher got a list of hospitals names that have family planning unit from the primary care department/ Ministry of Health.
2. Researcher has a frame list of (401) nurses names who work in obstetric ward, delivery room and maternity operational room and family planning clinics in six Baghdad City hospitals that were chosen according to having family planning clinic there.
3. Research consisted of 100 nurses as size of study sample. Researcher calculated the sampling interval as standard distances between the elements chosen for the sampling.
4. The first element was selected randomly.
5. The total sample was illustrated in (Table 1).
6. The period of data collection for all hospitals was two months. The research study was conducted in six hospitals at Baghdad City which include family planning clinic: Al-Elwia Maternity Teaching Hospital, Fatima Al-Zahra 'a Maternity and Pediatric Teaching Hospital, AL-Yarmouk Teaching Hospital, AL-Karkh maternity Hospital, AL-Kademya Teaching Hospital and AL-Nuaaman Teaching Hospital. Nurses who work in obstetric ward, delivery room, obstetric operational room in their hospitals and family planning units were selected as study sample.

Table 1. Selection of study Sample According to Systematic Random Sampling from Six Hospitals in Baghdad City.

No.	Hospitals Name	Total Number	Sample Size
	AL-Karkh maternity Hospital	71	18
	Al-Elwia Maternity Teaching Hospital	106	26
	Fatima Al-Zahra'a Maternity and Pediatric Teaching Hospital	91	23
	AL-Kademya Teaching Hospital	43	11
	AL-Yarmouk Teaching Hospital	65	16
	AL-Nuaaman Teaching Hospital	25	6
Total		401	100

A questionnaire was used as a tool of data collection to fulfill with objective of the study and consisted of four parts, including demographic, reproductive characteristics, personal experience in using method or in attending continuing education training and source of knowledge. Pilot study was carried out between the February 2nd to February 21 of 2012, on (10) nurse who work in maternity department to determine the reliability of questionnaire and content validity was carried out through the 17 experts. Descriptive statistical analyses were used to analyze the data. Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 16, and Excel. Through the application of descriptive statistical data analysis includes (Frequencies, Percentage, Mean, Standard Deviation & Mean score). All questions rated according to the following criteria: Yes= 2, No= 1.
So the cut-off-point = 1.5

RESULTS

(Table 2) Shows that the highest percentage (43%) of study sample at age group (40-49) years while the lowest percentage (13%) of them between age (20-29) years and the same at (55-59) years; and the mean age with SD of age: (39.76± 8.41). The highest percentage (39%) of study sample was graduated from secondary school of nursing while the lowest percentages (4%) were college of nursing. The highest percentage (53%) of study sample work at obstetric and gynecologic word, while lower percentages were (2%) work at family planning clinic. The highest percentages (84%) were married and (16%) were single.

Table 2. Distribution of Study Sample According to Demographic Characteristics.

Demographic Characteristics	Nurses (n=100)	
Age/years	No.	%
20 - 29	13	13%
30 - 39	31	31%
40 - 49	49	49%
50 - 59	13	13%
$\bar{x}$ ± SD 39.76± 8.41		
Educational Level		
Nursing school graduated	16	16%
Secondary school nursing graduated	39	39%
Secondary school midwifery graduated	25	25%
Institute of medical technology/nursing	15	15%
College of nursing	5	5%
Location of Work		
Ward	53	53%
Delivery room	32	32%
Theater room	13	13%
Family planning clinic	2	2%
Marital Status		
Single	16	16%
Married	84	84%

(Table 3) shows The highest percentage (70.2%) of study sample had three and more pregnancies, while (20.2%) of them nulligravida. The highest percentage (55.9 %) of study sample had three and more deliveries while the lowest percentage

(9.5%) was nullipara. The highest percentage (51%) of study sample had three and more, while (9.5 %) of them hadn't child. Regarding abortion: The highest percentage (48.8%) of study sample had no history of abortion, while (3.5%) of them had five and more abortions.

Table 3. Distribution of Study Sample According to Reproductive Characteristics

Reproductive Characteristics	Nurses (n=84) No.	%
Gravidity		
*nulligravida	8	9.5%
1-2	17	20.2%
(≥3)	59	70.2%
Parity		
*nullipara	8	9.5%
1-2	29	34.5%
(≥3)	47	55.9%
Alive Children		
*None	8	9.5%
1-2	33	39.2%
(≥3)	43	51%
Abortion		
*None	41	48.8%
1-2	34	40.4%
3-4	6	7.1%
(≥5)	3	3.5%

* *(84) nurses in study of nurses were married*

(Figure 1): shows the highest percentage (44%) their sources of knowledge about natural family planning were from Friends and family.

The highest mean of score (1.86) in knowledge regarding item No. (7) Which refer to effectiveness of LAM is (98%), while the lowest mean of score (1.2) was in item No. (1) Which refer the effectiveness of SDM is (95%). So the grand mean score (1.37) for all methods (Table 4).

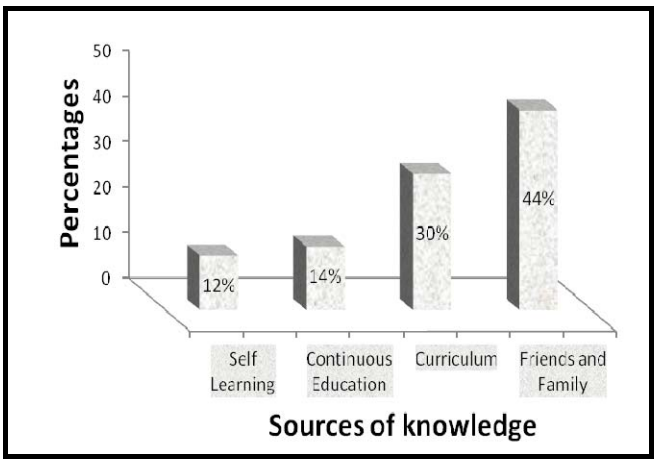

Figure1. Sources of Nurses Knowledge Regarding Natural Family Planning Methods.

Table (4): Distribution of Nurses by Knowledge about the Effectiveness of Natural Family Planning Methods.

NO.	Perfect Use Effectiveness Natural Family Planning Methods in to Avoiding Pregnancy	Nurses (n=100) Yes No.	Yes %	No No.	No %	M.S
1	Standard day method is (95%)	20	20%	80	80%	1.2
2	Calendar-rhythm method (92%)	34	34%	66	66%	1.34
	Two-Day method is (96%)	18	18%	82	82%	1.18
	Basal body temperature (BBT) method (99%)	36	36%	64	64%	1.36
	Symptothermal method (98%)	34	34%	66	66%	1.66
	Ovulation method is (97%)	37	37%	63	63%	1.37
	Lactational amenorrhea (98%)	86	86%	14	14%	1.86

Grand mean score = 1.37

DISCUSSION

The highest percentage (43%) of study sample at age group (40-49) years while the lowest percentage (13%) of them between age (20-29) years and the same at (55-59) years; and the mean age with SD of age: (39.76± 8.41) as shown in Table [1]. This finding is consistent with study of

Moura et.al [8] reported that out of 121 nurses from health system of Fortaleza,CE, Brazil, their range age (26-59) years, while the average (38.3) years. And the highest percentage (39%) of study sample was graduated from secondary school of nursing while the lowest percentages (4%) were college of nursing. This finding is consistent with study of Fehring [9] who reported that the (80%) of study sample are graduated from basic nursing program. Nurses are person who qualified to educate and counsel their couple about NFP method. So they need to be educated and have a lot of knowledge regarding NFP method to be good counselor[10]. Nurses have been well known as patient educators. They are known to empower patients with information regarding health issues and concerns. Nurses don't just tell patients what to do, but also why and how [11].

The finding of present study is consistent with study of Moura who reported (51.1%) out of 121 nurses have children while (48.9%) not have children[8].

The highest percentage (44%) of them their sources of knowledge were from friends and family, while the lowest percentage (12%) by self learning. The finding is inconsistent with Fehring who reported that source of learned NFP method (44% vs. 24%) from training, (29% vs.19%) from continuous education and (52% vs. 63%) by self-instruction[12]. (Table 2) shows that nurses had acceptable knowledge regarding effectiveness of perfect use of Lactational amenorrhea and Symptothermal method, while had unaccepted knowledge regarding of perfect use of effectiveness of the following methods: Standard day method, Calendar-rhythm method, Two-day method, Ovulation method and Basal body temperature. The finding of this study is consistent with Fehring in his study faxed questionnaires on natural family planning to a random sample of 317 active physicians and all gynecological registered with college of physician and surgeons of British Colombia and all (N=239) family medicine and gynecologist at university of British Colombia. They obtained a 44% return rate from both groups. When asked about the effectiveness of natural family planning method to avoid pregnancy, 6% were correct identifying perfect use of methods, and 33% were correct in estimating the typical use effectiveness of natural family planning methods[12]. So nurse and other health care professionals often have little knowledge of methods of natural family planning and do not readily prescribe natural method for their couple, one reason for this is that little or no information on natural family planning is provided in nursing or medical school [13].

CONCLUSION

Based on study findings; nurses had inadequate knowledge regarding the effectiveness of different type of natural family planning because of not having awareness about these methods and not having equability source of knowledge about natural family planning.

RECOMMENDATION

Increase nurse's awareness about effectiveness and type of natural family planning methods by continuous education.

Distribute scientific booklet about natural family planning methods and its effectiveness.

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پوختە

زانیاریا تیمارکاران لدور بکێرهاتنا رێکێن سروشتی بو رێکخستنا خێزانی

پێشەکی و ئارمانج: رێکێن سروشتی بو رێکخستنا خێزانی دهێنە هژمارتن وەك رێکێن بسانەهی و بێ زیان بو نەهێلانا دووگیانیا نەڤیای. تیمارکاران رولەکێ گرنگ یێ هەی د ئاموژگاریکرن و رەوشەنبیرکرنا ئافرەتان دا دژیێ بەرهەڤ بو دووگیانیێ.

ئارمانجا ڤەکولینێ هەلسەنگاندنا ئاستێ زانیاریا تیمارکارانە لدور بکێرهاتنا رێکێن سروشتی بێن رێکخستنا خێزانی بو نەهێلانا دووگیانیا نەڤیای.

رێکێن ڤەکولینێ: ڤەکولینەکا ساخلەتی هاتە کرن و نمونەکێ بهەروە ژ 100 تیمارکاران بەشداربوون کو کار دکەن ل بەشێن ژنان وزاروکبوونێ ل شەش نەخوشخانێن باژێرێ بەغدا. ئەڤان نەخوشخانا کلینیکا رێکخستنا خێزانی تێدا هەبوو.ڤەکولین هاتە ئەنجامدان هەر ژ 24ێ شواتێ 2012 هەتا 25ێ نیسانا 2012. پرسنامەك هاتە ئامادەکرن بو ڤەکولینێ کو چار پارچە بخوڤە گرتبوون: پێزانینێن کەسایەتی، ساخلەتێن زاییناێ، بکارئینانا رێکێن سروشتی بێن رێکخستنا خێزانی یان بەشداربوون درەوشەنبیرکرنێ دا لدور رێکێن سروشتی. لدەستپێکێ پرسنامە هاتە بکارئینان وەك تێست بو هەلسەنگاندنا پرسنامێ.

ئەنجام: پرانیا دایکان تێکرایێ ژیێ وان 39 سال بوو و 39٪ ژ بەشداربوویا دەرچوویێن قوتابخانێن دواناوەندی بێن تیمارکاریێ بوون. سەرجەمێ 53٪ ژوان کار دکرن ل بەشێ ژنان وزاروکبوونێ و 84٪ د شی کری بوون. رێژا مەزن ژوان (44٪) ژێدەرێ پێزانینێن وان لدور رێکێن سروشتی هەڤال و خێزان بوون. ئاستێ زانیاریێ لدور بکێرهاتنا رێکێن سروشتی یێ گەلەك ژوان نەیێ باش بوو و ئەگەرێ سەرەکی ئەوە کو پێزانین کێم وەرگرتینە و لاوازیا ژێدەرێ پێزانینا.

دەرئەنجام: زانیاریا تیمارکاران لدور بکێرهاتنا رێکێن سروشتی بو رێکخستنا خێزانی یا کێمە ژبەر کو دروست نەهاتینە رەوشەنبیرکرن لدور بابەتی و ژیدەرێ وان بو پێزانینا یێ لاوازە.

الخلاصة

معارف الممرضات عن فاعلية طرق تنظيم الأُسرة الطبيعية

أهمية الدراسة: تعتبر الطرق الطبيعية لتنظيم الأُسرة من الطرق السهله وليس لها تأثيرات جانبيه عند استخدامها في منع الحمل غير المرغوب به علما بان للممرضة دور مهم في تقديم المشورة والتثقيف الصحي للأمهات في سن الإنجاب.

الهدف: تقييم معارف الممرضات عن فاعليه الطرق الطبيعية لتنظيم الأُسرة في مستشفيات مدينه بغداد.

المنهجية: اجريت دراسة وصفية على عينة احتمالية (عشوائية منظمة) من (100) ممرضة يعملن في قسم النسائية والتوليد في ست مستشفيات في مدينة بغداد التي تحتوي على عيادة تنظيم الأُسرة وتشمل: مستشفى الكرخ للولادة, مستشفى العلوية التعليمي للولادة, مستشفى فاطمة الزهراء التعليمي, مستشفى اليرموك التعليمي, مستشفى الكاظمية التعليمي, مستشفى النعمان التعليمي. انجزت الدراسة للفترة من 22 شباط 2012 إلى 25 نيسان 2012, استخدمت الاستبانه كأداة لجمع المعلومات لتحقيق هدف الدراسة وتتكون من أربعة أجزاء تتضمن الخصائص الديموغرافية, الانجابية, , مصدر المعلومة عن الطرق الطبيعية لتنظيم الأُسرة ومعلوماتهن عن فاعلية الطرق الطبيعية لتنظيم الأُسرة. تم اجراء الدراسة الاستطلاعية لاختبار ثبات الاستبانة وجرى صدق المحتوى من خلال (17) خبير واستخدام الإحصاء الوصفي في تحليل البيانات.

النتائج: أظهرت النتائج إن معظم الممرضات معدل أعمارهن (39) سنة و(39٪) من هن خريجات أعدادية تمريض و (53٪) يعملن في ردهات النسائية والتوليد. و (84٪) منهن متزوجات. اما عن مصدر معلوماتهن عن الطرق الطبيعيه لتنظيم الأُسرة فكانت أعلى نسبه (44٪) من الاهل والاصدقاء. تبين الدراسة ان معظمهن لا يمتلكن معارف مقبولة عن فاعليه الطرق الطبيعية لتنظيم الأُسرة ومن احد هده الاسباب قلة المعلومات عن هده الطرق في المنهاج التمريضي و ايضا بسبب عدم رصانة مصدر معلوماتهن عن هذه الطرق.

الاستنتاجات:استنادا الى نتائج الدراسة, ان الممرضات لا يمتلكن معارف مقبولة عن فاعليه الطرق الطبيعية المختلفة لتنظيم الأُسرة وقلة وعيهن و عدم رصانة مصدر معلوماتهن عن مختلف طرق تنظيم الاسرة الطبيعية.

التوصيات: أوصت الدراسة بتعزيز معارف الممرضات عن الطرق الطبيعية لتنظيم الأُسرة بواسطة دورات التعليم المستمر وتصميم كتيب عن هده الطرق لدورهن الفاعل في تقديم المشورة للأزواج في المجتمع لكون هده الطرق خاليه من الإضرار الجانبية التي قد تسببها الطرق الهرمونية (حبوب منع الحمل) أو الميكانيكية (اللولب) فضلا عن نسبة فاعليتها العالية في منع الحمل غير المرغوب به فيما اذا استعملت بالطريقة الصحيحة.

DOCTOR'S KNOWLEDGE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN HOSPITALS OF DUHOK PROVINCE

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ABSTRACT

Background: Awareness of the risks of colonization and subsequent infection, effective treatment and the appropriate use of antibiotic prescribing remain as high priorities to combat Methicillin-resistant Staphylococcus aureus (MRSA) related issues.
Objectives: The aim of this study was to assess the knowledge of doctors working in the hospitals of Duhok province about MRSA.
Methods: the study was done in 8 hospitals of Duhok province. The participants were doctors working in those hospitals with any professional qualification. A questionnaire of ten true and false questions was developed to study their knowledge of MRSA related issues.
Results: A total 239 doctors filled the questionnaire. Nearly two thirds of them were in the age group of 24-34 years, with a male to female ratio of 3.4:1. The majority of respondents (90.4%) correctly identified MRSA as a Gram-positive organism. 72% of them identified the correct anatomical sites for MRSA colonization and 68.2% realized that MRSA could be acquired in the community without prior hospitalization and 41.4% of doctors thought that Cefoxitin is the drug of choice for MRSA. The rate of correct answer was significantly higher in male than female doctors. Age, being a specialist, type of specialty and type of hospital did not affect the knowledge of doctors significantly.
Conclusion: doctors working in hospitals of Duhok province have good knowledge about MRSA, but in some areas of the subject, they have low or no information. This necessitates a well-defined program for control of hospital-acquired infections including MRSA.

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Key words: Doctor's Knowledge ,MERSA, Duhok

Staphylococcus aureus has long been recognized as an important pathogen in human diseases. Methicillin-resistant Staphylococcus aureus (MRSA) is the name given to a strain of the Staphylococcus aureus bacteria that is resistant to a number of antibiotics. MRSA is a major cause of hospital-acquired infections that are becoming increasingly difficult to combat because of emerging resistance to all current antibiotic classes.[1,2] According to The European Centre for Disease Prevention and Control, in 2008 MRSA accounts for 44% of nosocomial infections, 22% of attributable extra deaths and 41% of extra days of hospitalization associated with these infections.[3] MRSA inhabits the nose and some other body sites of normal individuals without causing any disease. In hospitalized patients, however, it can cause wound infections, pneumonia, septicemia and death. [4] Whereas hospital-acquired MRSA has been a concern for in-patients since the 1960s, the threat of community-acquired MRSA (CA-MRSA) has recently been associated with healthy people without traditional risk factors. [5,6]

Knowledge about health problems is crucial to deal with them properly and it is the first step in modifying behavior in relation to physicians' adherence to

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clinical practice guidelines. Awareness of the risks of colonization and subsequent infection, effective treatment and the appropriate use of antibiotic prescribing remain as high priorities to combat MRSA related issues. [7,8]

To the best of our knowledge, no MRSA awareness survey targeting doctors and other medical staff has been performed in Duhok province. The aim of this study was to assess the knowledge of doctors working in the hospitals of Duhok province about MRSA which would be as the first step of further projects and studies. We also aimed to identify specific doctors' demographics and variables associated with their knowledge levels to MRSA related issues.

METHODS

The study was a cross sectional one done in hospitals of Duhok province. Duhok is one of the three provinces of Kurdistan Region of Iraq. There were eight hospitals in Duhok province. Three of those were teaching hospital situated in Duhok city including. Azadi general hospital, Heevi pediatrics hospital and Duhok emergency hospital. The others were Zakho general hospital in Zakho district, Gulan hospital and Akre emergency hospital in Akre district, Amedi general hospital in Amedi district and Duhok burn and plastic surgery hospital in Duhok city. The overall number of doctors working in these hospitals was 549 at the time of data collection.

A questionnaire was developed to study the knowledge of doctors working in those hospitals about MRSA. The questionnaire included ten true and false questions, they were about the nature of MRSA; risk factors for MRSA, common sites of colonization, screening, prevention, control and treatment. The questions were inferred mostly from the guidelines for the control and prevention of MRSA in healthcare facilities. [8] Demographic data were also covered by the questionnaire including age, gender, credential, specialty, professional qualification and the working place (hospital).

A convenience sample was recruited from all the eight hospitals. The inclusion criteria for participants were a medical doctor, who is currently working in one of the eight hospitals. Data collection from each hospital was conducted in one single day, except for Azadi general teaching hospital, the largest one and with the highest number of staff, in which the survey was conducted in two days. On the day of data collection, all departments of the hospital were visited and every doctor who was available at the time of visit was participated. Questionnaire was filled by the participants themselves in front of one of the investigators after short clarification of the nature of the survey and obtaining consent, without explaining the specific questions to them. Data collection for this study was completed within nine days in February, 2011.

The survey results were analyzed using SPSS v.18. Description of demographic data and frequencies of correct and incorrect answers were performed through frequency tables and differences between groups were compared using Chi-squared test with a 5% significance level.

RESULTS

A total of 241 doctors were interviewed. The response rate was (99.2%) as only two of them refused to participate. Nearly two thirds of them (61.1%) were in the age group of 24-34 years, with a male to female ratio of 3.4:1. About one third (33.5%) were specialists, while the rest (66.5%) were either house officers, or practitioners. The vast majority of the interviewees (73.2%) were from the three teaching hospitals. Participants were from different specialties and different medical departments of hospitals. (Table 1)

The majority of respondents (90.4%) correctly identified MRSA as a Gram-positive organism. 72% of doctors

identified the correct anatomical sites for MRSA colonization and 68.2% realized that MRSA could be acquired in the community without prior hospitalization. The lowest rate of correct answers (44.4%) was to statement number five which was about the transfer of patient in between hospital wards, followed by statement number four about how to reduce MRSA transmission (56.5%) and 41.4% of doctors thought that Cefoxitin is the drug of choice for MRSA (Figure 1).

Table 1 .Demographic characteristics of the study population (n=239)

Demographic variable		Number	%
Age (years)	24 – 34	146	61.1
	35 – 45	60	25.1
	> 45	33	13.8
Gender	Male	185	77.4
	Female	54	22.6
Professional qualification	Non-specialists	159	66.5
	Specialists	80	33.5
Type of Hospital	Teaching	175	73.2
	Non-teaching	64	26.8
Department and/or specialty	Internship	62	25.9
	Medicine	28	11.7
	Surgery	44	18.4
	Gynecology and Obstetrics	15	6.3
	Pediatrics		
	Ophthalmology	22	9.2
	ENT	11	4.6
	Dermatology	7	2.9
	Orthopedics	6	2.5
	Radiology	6	2.5
	Laboratory	15	6.3
	General Practice	11	4.6
		12	5.0

Table (2). Association of some variables with knowledge

Variable		Low knowledge 1-5 correct answers No. (%)	Good knowledge 6-10 correct answers No. (%)	P value
Age (years)	24-34	22 (15.2)	123 (84.8)	NS*
	35-45	9 (15)	51 (85)	
	> 45	4 (12.1)	29 (87.9)	
Gender	Male	22 (11.9)	163 (88.1)	0.026
	Female	13 (24.1)	41 (75.9)	
Professional Qualification	Not a specialist	25 (15.6)	135 (84.4)	NS
	Specialist	10 (12.7)	69 (87.3)	
Type of specialty	Medical	5 (12.5)	35 (87.5)	NS
	Surgical	5 (12.8)	34 (87.2)	
Type of Hospital working in	Teaching	25 (14.3)	150 (85.7)	NS
	Non teaching	10 (15.6)	54 (84.4)	

** NS = not significant (p-value > 0.05) using Chi-squared test*

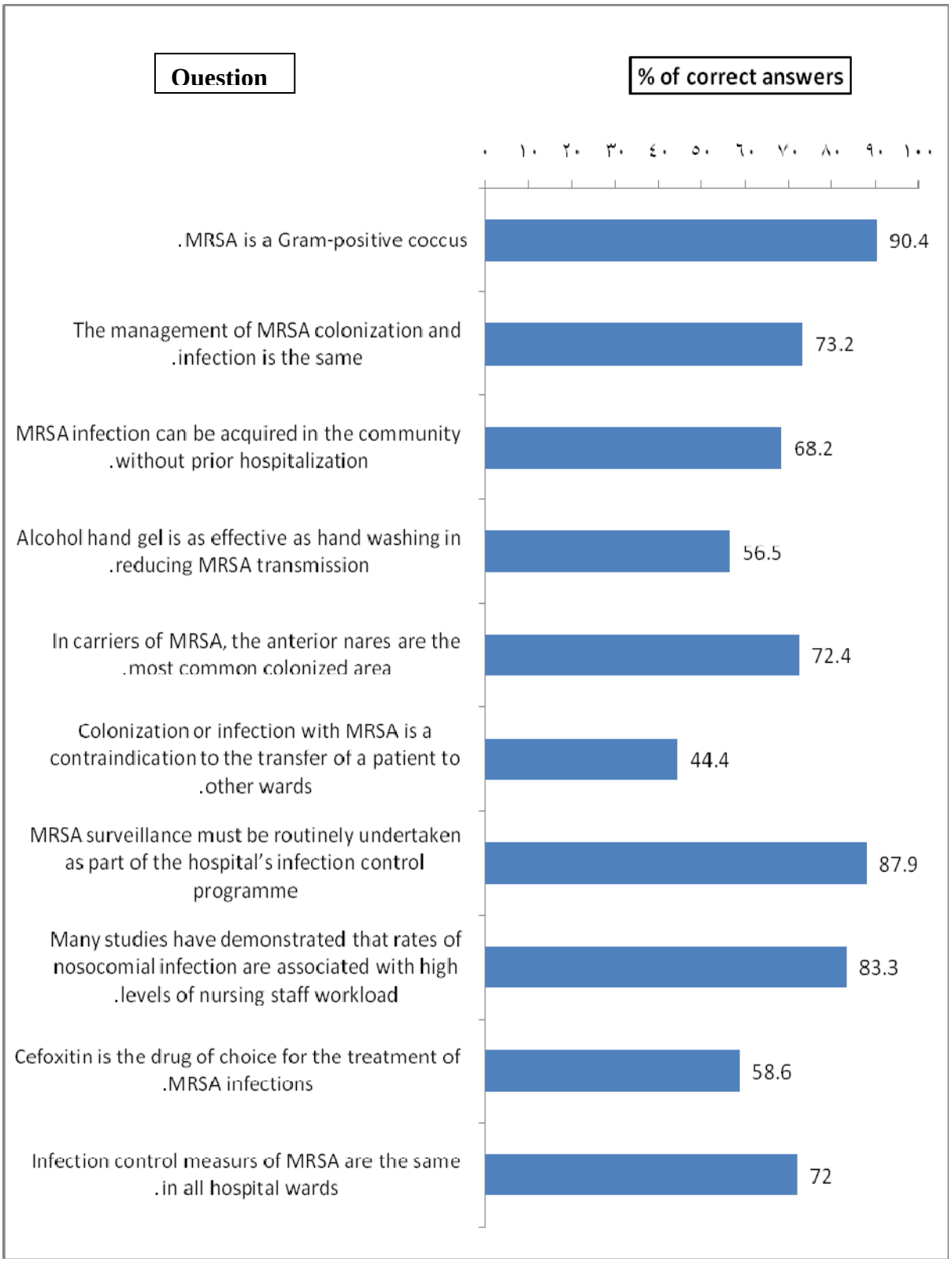

Figure 1. Proportion of participants providing the correct response for each question

For the purpose of comparisons, the knowledge of doctors about MRSA was categorized into two categories, low knowledge (those who had correctly answered five or less statements) and good knowledge (those who had six and more correct answers). It was shown that with increasing age, the rate of correct answers was numerically, but not statistically, higher (Table 2). The rate of correct

answer was significantly higher in male doctors (88.1%) than female doctors (75.9%) with a p-value of (0.026). Being a specialist did not affect the knowledge of doctors, though the specialist had slightly more knowledge than non-specialist (87.3% compared to 84.4%). Amongst specialists, there was no difference between those with medical background compared to those with surgical background (Table 2). Finally, no significant difference was found in responses obtained from doctors working in teaching hospitals and those working in non-teaching hospital (85.7 and 84.4% respectively).

DISCUSSION

Cross sectional studies have their own limitations. Despite that, however, they are essential in providing basic data, especially in the case of MRSA when no such data is available in the region. Doctors were chosen as participants for this study without involving other health staff as no special guidelines and systems for control of infections inside hospitals are available for the hospital staff. In some countries were such guidelines are available, they usually assess the knowledge of all health staff in addition to that of patients and even of the general public. [4,7,8,10,11] The design of the questionnaire was true and false statements. This type of questionnaire was also performed in a study done in United Kingdome. [8] This could be one of the limitations of this study as the participants guessed the answer to some questions. Eventually, this affected the real level of their knowledge about MRSA.

The interviews were conducted in all hospitals, except one, in a one day visit. The aim was to limit the chance of sharing knowledge about the subject between doctors. The purpose of having the questionnaires completed at the time of interview was so that answers were spontaneous, without the use of reference materials such as textbooks or internet websites. The sample size of 239 was thought to be good as it represent nearly 44% of the total number of doctors' population in the hospitals of Duhok province. A higher rate of males than females was due to the fact that the total number of female doctors constituted only 35% of the total number of doctors in the involved hospitals.

Majority of participants realized that MRSA is a Gram-positive organism, and this was convenient with the answer to the same question in a study done in Scotland [7.] More than half of the doctors in this study (55.6%) agreed that it is contra-indicated to transfer patients colonized or infected with MRSA between hospital wards. This gave us an idea that participants over estimated the danger of MRSA. Such strict attitude toward isolation of MRSA patients was also seen among staff of ENT and general surgery wards in a study done in England. [12] The low rate of correct answers to statement nine indicated that participants had low knowledge of what antibiotics MRSA is resistant to; 41.4% of them thought that cefoxitin is the drug of choice for the treatment of MRSA, which is in fact one of the antibiotics that MRSA is resistant to.

The study showed that male have more knowledge of MRSA than female doctors. The reason is difficult to be explained and is against the results stated in the study done in United Kingdome when no difference was found between the two genders.[8] No significant differences were detected regarding all other studied variables including age, professional qualification, type of specialty and type of the hospital. This might be due to the fact that no organized program of hospital acquired infection control was applied in any of the involved hospitals and the subject of MRSA was not touched in the continuing medical education activities.

In conclusion, doctors working in hospitals of Duhok province have good knowledge about MRSA in some areas of the subject that is beside low or no information

regarding other aspects. This necessitates a well-defined program for surveillance and control of nosocomial infections including MRSA and to tackle this issue in continuing medical education activities.

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پوختە

زانیاریا نوژداران ل نەخوشخانێن دهوکێ لدور بەکتیریا بەرگر بو دەرمانێن زیندەدژ

پێشەکی: ئاگەهداری لدور مەترسیا هەلگرتن و هەودان ب بەکتیریا بەرگر بو دەرمانێن زیندەدژ هەروەسا چارەسەری و دروست بکارئینانا دەرمانێن زیندەدژ ژ ئارمانجێن سەرەکی یە بو دژاتییا ڤێ بەکتریایێ.

ئارمانج: هەلسەنگاندنا ئاستێ زانیاریا نوژدارێن نەخوشخانێن دهوکێ لدور بەکتیریا بەرگر بو دەرمانێن زیندەدژ.

ریکێن ڤەکولینێ: ئەڤ ڤەکولینە ل هەشت نەخوشخانێن پارێزگەها دهوکێ هاتە ئەنجامدان. بەشداربوویێن ڤەکولینێ نوژدار بوون کو ل ڤان نەخوشخاان کاردکر. پرسنامەك کو ژ دەھ پسیارێن بەرسڤ ب دروست یان نە هاتە ئامادەکرن بو هەلسەنگانا ئاستێ زانیاریێ.

ئەنجام: دسەرجەم دا 239 نوژدار بەشداری ڤەکولینێ بوون. نێزیکی دوو سێیتکا دژیێ 24–34 سالی دا بوون و ریژا نوژدارێن نێر بو یێن مێ 3.4:1 بوو. پرانیا وان (90.4٪) دزانی ئەڤ بەکتیریایە چ جورە. 72٪ دروست بەرسڤ دا بوو لسەر جهێ لەشی کو مروڤ بەکتیریایێ لێ هەلگریت و 68.2٪ باوەردکر کو ئەڤ بەکتیریایە دناڤ جڤاکی دا ژی یا هەی لدەف کەسێن نەهاتینە نڤاندن ل نەخوشخانا. 41.4٪ هزر دکر کو سیفوگزیتین دەرمانێ هەلبژارتییە بو چارەسەریێ. ریژا بەرسڤێن دروست پتر بوو لدەف نوژدارێن نێر، لێ ژیێ نوژداری، تایبەتمەندی و جورێ تایبەتمەندیێ، و جورێ نەخوشخانێ هیچ کارتێرن لسەر زانیاریا نوژداران نەکربوو.

دەرئەنجام: نوژدارێن نخوشخانێن دهوکێ زانیارییەکا باش یا هەی سەبارەت بەکتیریا بەرگر بو دەرمانێن زیندەدژ، هەر چەندە دهندەك واران دا پێزانین دکێمن یان هەر نینن، وئەڤە گرنگیا هەبوونا بەرنامەکێ ریکخستی یێ کونترولکرنا کولبوونێن ل نەخوشخانێ دهێنە وەرگرتن ددەت.

الخلاصة

دراية الأطباء في مستشفيات محافظة دهوك بموضوع البكتريا المقاومة للمضادات الحيوية MRSA

خلفية وأهداف البحث: ان الادراك بمخاطر وجود البكتريا في الجسم أو الاصابة بها والعلاج الفعال مع الاستعمال السليم للمضادات الحيوية تبقى الأولوية لمقاومة بكتريا MRSA. ان الهدف من البحث كان تقييم مستوى معرفة ودراية الأطباء العاملين في مستشفيات محافظة دهوك حول موضوع ال MRSA.

طرق البحث: أجري البحث في مستشفيات محافظة دهوك الثمانية والمشاركون كانوا الأطباء العاملين في تلك المستشفيات بمختلف مراحل التدرج والاختصاص. تم تحضير استبيان مكون من عشرة أسئلة تكون الاجابة عليها ب صحيح أو خاطىء تتعلق بموضوع البكتريا المقومة للمضادات الحيوية.

النتائج: كان مجموع الأطباء المشاركين 239 طبيب. حوالي ثلثين منهم كانوا من الفئة العمرية 24–34 سنة و كانت نسبة الذكور الى الاناث 3.4:1. أغلب المشاركين (90.4٪) عرفوا بأن البكتريا هي موجبة لصبغة كرام. نسبة 72٪ منهم كانوا على دراية بمكان استيطان البكتريا في الجسم و 68.2٪ منهم أدركوا بامكانية تواجد البكتريا في المجتمع بين من لم يتعرضوا لبيئة المستشفى، ونسبة 41.4٪ منهم كانوا يظنون بأن السيفوكزيتين هو الدواء المختار لعلاج هذه البكتريا. نسبة الاجابات الصحيحة كانت أكثر لدى الأطباء الذكور مقارنة بالاناث، بينما العمر والاختصاص و نوع المستشفى لم تؤثر على مستوى معرفة الأطباء بالموضوع.

الاستنتاج: ان للأطباء العاملين في مستشفيات محافظة دهوك معرفة جيدة بموضوع البكتريا المقاومة بصورة عامة مع انها قليلة في جوانب معينة من الموضوع، وهذا يتطلب استحداث برنامج متطور للسيطرة على الأمراض المعدية داخل المستشفى بما يتضم البكتريا المتعلقة بهذا البحث

EXTRACTION OF PURE KETAMINE POWDER AND STUDY THEIR ANALGESIC EFFECT AS A GEL ON MICE USING A HOT –PLATE TEST

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ABSTRACT

The present study was undertaken to extract ketamine powder from ketamine hydrochloride by precipitate ketamine. After that we examine the purity of this powder by infra-red (FTIR) and ultra-violet(UV) spectroscopy. ketamine gel in different concentrations was prepared (0.5 , 1 , 5 , 10 , 15)% to evaluate the antinociceptive activity. ketamine powder was seen is pure and this show in infra-red and ultra-violet scanner. Ketamine gel at concentrations 0.5, 1, 5, 10,15) % produce antinociceptive in mice (5.6±2.2) (4.4±2.0) (8.2±4.3) (10.6±5.2) (8±2.1) second after 2 min respectively by using a hot plate test in comparison with control(2.4±2). The percentage of maximum possible effect (MPE) increased from (9.9) % in control group to (23.3) (18.3) (34.2) (44.2) (33.3)% respectively according to the concentrations of ketamine gel after 2 min . Purification of ketamine powder from ketamine solution and use as a gel to could be of value relief pain by topical application.

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Key words: Purification of ketamine, Ketamine gel , analgesia, Infrared spectroscopy, Hot-plate test

Ketamine, a phencyclidine (PCP) analog, has been used for more than 30 years to produce "dissociative" anesthesia [1]. In this state the patient is awake and can respond to stimuli but has a diminished sense of awareness and an amnesia for events occurring while under the influence of ketamine.Early experience with ketamine revealed that it also produced analgesia that sometimes well outlasted its anesthetic effects. Although the mechanisms of ketamine's analgesic effects remain the subject of debate, and are likely multiple [2-4]. Antagonism at the NMDA-receptor site appears to be central to both its anesthetic and analgesic effects [5, 6]. Ketamine is available only I.M , I.V administration but it has been used orally. Ketamine is a dissociative anesthetic that is used to provide sedation and anesthesia in short surgical procedure, Patient may have adverse psychological effect including hallucinations, nightmares, delusion, dissociative reaction and schizophrenic form psychosis[7]. Ketamine is primarily used for the induction and maintenance of general anesthesia, usually in combination with a sedative. Other uses include sedation in intensive care, analgesia (particularly in emergency medicine), and treatment of bronchospasm. It has been shown to be effective in treating depression in patients with bipolar disorder who have not responded to other anti-depressants [8]. Pharmacologically, ketamine is classified as an N-Methyl D-Aspartat (NMDA) receptor antagonist [9]. Ketamine is an antagonist of N-methyl 1-D-Aspartate (NMDA) class of glutamate receptors which is largely responsible for it's anesthetic and behavioral effect effects [7] .NMDA inhibition produce catalepsy, consistent with the effect of ketamine administration. Ketamine also produces profound analgesia which seen to be at least partially mediated by **μ** - opioid receptor , in addition to it's binding to the phenylcyclidine binding site on the NMDA. ketamine is not frequently used for treatment of humans ,because it induces psychedelic episodes in patients ,especially adults, there are an increasing number of reports about patients that have become addicted to ketamine [10]. Among

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the latest innovations of the pharmaceutical industry is the technology of drug delivery that overcomes the disadvantages of oral administration, these effects include first-pass metabolism and adverse drug side effects [11] . An ultimate route of administration that by pass these events would offer the patient drug delivery through skin has been a promising concept for a long time because skin is easy to access , has a large surface area with fast exposure to circulating and lymphatic net works and the rout is noninvasive [12] .Therefore, the aim of the present study is to extract ketamine powder from ketamine hydrochloride solution and prepare ketamine gel with examine the analgesic effects of topical gel application in mice.

METHODS

Preparation of ketamine powder

10 ml of (1M) sodium bicarbonate was slowly added to 100 ml of the aqueous ketamine hydrochloride solution 5% (Alhukamma company) under continuous stirring until the pH of solution was close to 11, stirring was continued for one hour, and then the ketamine was precipitated. The solvent was eliminated by filtration and washed several times with distal water and then dried. The powder was studied by infra-red and ultra-violet spectroscopy in order to confirm the structure of the converted product.

Infrared Spectroscopy.

The infrared spectra recorded for prepared ketamine was examined by using Bruker Tensor 27 IR spectrophotometer (Germany) in the region (500-4000 cm) using KBr disc. This measurement was carried out in University of Mosul, College of Education, Iraq.

Electronic Spectra Measurement:

The measurement was carried out by using ethanol as a solvent with (1cm) diameter quartz cell by using Shimatsu-UV-Vis recording UV-1600 spectrophotometer (Japan). This measurement was carried out in Mosul University, Collage of Science, Department of Chemistry.

Preparation of ketamine gel

Ketamine gel was prepared (0.5, 1, 5, 10, 15)gm of ketamine powder in 100ml gel base to give a final concentration of (0.5%, 1%, 5%, 10%, 15%) with continuous mixing using Vortex device to prepare a homogenous gel. Gels were kept in plastic containers and store at room temperature.

Determination of Analgesic Activity of Ketamine Gel by Using a Hot –Plate Test:
Mice were divided into 2 main groups (A,B) each group were subdivided into six group with 5 animals per each group and the test was assessed by the hot plate method (13). The mice were treated topically with ketamine gel (0, 0.5, 1, 5, 10, 15) % respectively, on the planter area of the for and hind limb.
All the animals in group A were tested on hot plate after 1 minute while the mice in group B were tested after two minutes from topically application of the gel to determine the onset of action of gel. The mice were placed on top of hot plate of 55±1 0C. The time between placement and jumping or licking the hind paw was recorded as response latency.
The reaction time was recorded for control mice and for the animals treated with ketamine gel.
The percentage increase in reaction time was calculated by using the following equation :- (14)
% increase in reaction time (antinociceptive)=(T1-T0 / 30-T0) ×100
T0 = mean time for the control group (second)
T1 = mean time for the test group (second)
30= cut off time (second)

Statistical analysis

The data were expressed as mean ± SD , difference between three experimental

groups were statistically analyzed by one way analysis of variance (ANOVA) followed by the least significant difference test. The level of significance was at **p**≤0.05 . (15)

RESULTS

(Figure 1) represents the vibrational response of pure Ketamine when passed via an infrared beam. The spectrum showed band at 3000-3200cm-1 which attributed to the N-H stretch from the amide group connected to the cyclohexanone. The spectrum also showing band at 2800-2900 cm-1 which assign to C-H stretch from an alkyl group. At this frequency the alkyl group is generally a non aromatic CH3 or CH2 stretch. The band at 1750 cm-1 due to R2 – C=O stretch which appear very precise and typical stretch for cyclic ketones. In Ketamine the carbonyl is connected to the cyclohexane ring. The band 1600 cm-1 assigned to C-N band (Generally expressed in C-NH2 and C-N=O compounds).The band at 1400-1500 cm-1 is attributed to C-H bend, this is another vibration mode of the CH2 or CH3 components of Ketamine. This is not the C-H bond from the aromatic carbons. The band at 1450cm-1 region is due to C-C stretch. This carbon to carbon stretch is not for the aromatic specie and hence characterizes the bonding involved in the cyclohexane ring. Pure ketamine extract measurement by UV (Ultra-Violet) spectroscopy (Figure 2).

Analgesic effect of ketamine Gel:

The results of assessment of analgesic activity of ketamine gel at (0.5 , 1 , 5 , 10 , 15)% shown that the gel has no significant difference after 1min from application of gel between control(2.4±2.6) second and all treated groups(1.4±1.6)(2.4±2.4)(5.8±5.4)(4.6±4)(6.2±3.1) second respectively at **p**≤0.05.(Table 1),(Figure 3).

The result shown that the application of ketamine gel after 2min produce a highly significant difference between groups treated with ketamine gel at concentration (5, 10, 15) % (8.2±4.3) (10.6±5.9) (8±5.5) second respectively in comparison with control(2.4±2) second and other treated group (Tables 2 and 3).

Topical application of ketamine gel at 0.5% and 10% produce analgesic activity in highly significant difference after 2min (5.6±3.5) (10.6±5.9) sec at **p**≤0.05 in comparison with same concentration after 1min from application (1.4±1.6)(4.6±4)sec respectively at **p**≤0.05(Table 2)(Figure 4).

Reaction time (antinociceptive) was increased from (10)% in control treated group after 1min to (25, 19.2, 25.8)% according to the concentration of gel (5, 10, 15)% (Figure 2), while after 2min the percentage of reaction time (antinociceptive) increase to (23.3, 18.3, 34.2, 44.2, 33.3)% respectively according to the increase concentration of ketamine gel(0.5, 1, 5, 10, 15) in comparison to control group (9.9) % (Figure 5).

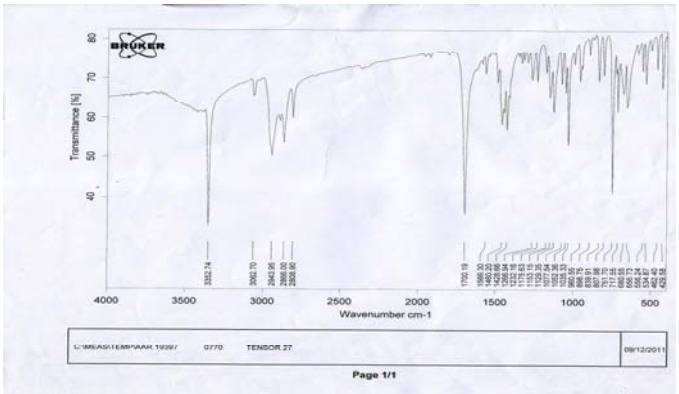

Figure 1. Pure ketamine extract measurement by FTIR (Infra-red) spectroscopy .

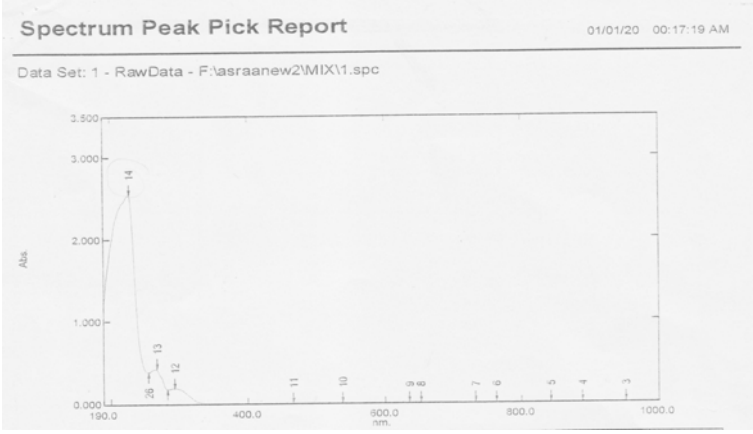

Figure 2. Pure ketamine extract measurement by UV (Ultra-Violet) Spectroscopy.

Table 1. Statistics of antinociceptive effect of ketamine gel after 1 minutes

ANOVA After 1 Minute

	Sum of Squares	Df	Mean Square	F–value	*p*–value
Between Groups	100.400	5	20.080	1.707	0.171
Within Groups	282.400	24	11.767		
Total	382.800	29			

Table 2. Statistics of antinociceptive effect of ketamine gel after 2 Minutes.

ANOVA after 2 Minutes

	Sum of Squares	Df	Mean Square	F–value	p–value
Between Groups	219.867	5	43.973	2.503	0.048*
Within Groups	421.600	24	17.567		
Total	641.467	29			

*Indicated significant difference at $p \leq 0.05$.

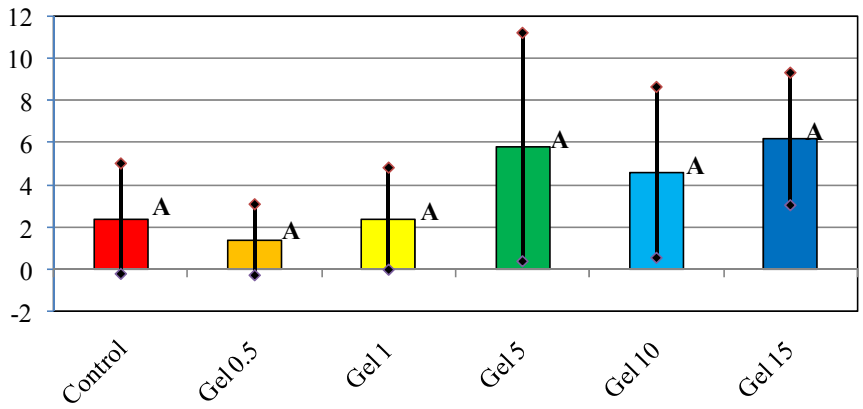

Figure 3. Mean ±S.D of antinociceptive effects of ketamine gel after 1minute in hot-plate test.

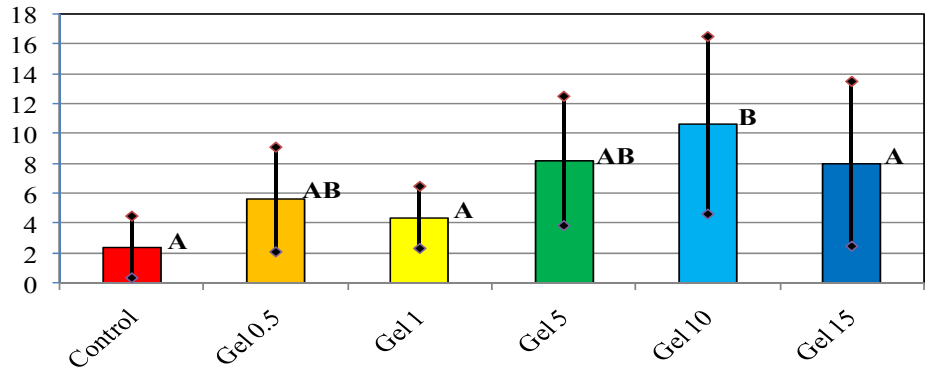

Figure 4. Mean ±S.D of antinociceptive effects of ketamine gel after 2minutes in Hot-plate test.

Table 4. Antinociceptive effect of different concentration of ketamine gel after 1 and 2 min Hotplate Student's t–test

Concentration	Time	No.	Mean	± SD	t–value	df	*p*–value
Control	After 1 Minute	5	2.40	2.608	0.000	8	1.000
	After 2 Minutes	5	2.40	2.074			
0.5%	After 1 Minute	5	1.40	1.673	–2.417	8	0.042*
	After 2 Minutes	5	5.60	3.507			
1%	After 1 Minute	5	2.40	2.408	–1.407	8	0.197
	After 2 Minutes	5	4.40	2.074			
5%	After 1 Minute	5	5.80	5.404	–0.775	8	0.460
	After 2 Minutes	5	8.20	4.324			
10%	After 1 Minute	5	4.60	4.037	–1.868	8	0.05*
	After 2 Minutes	5	10.60	5.941			
15%	After 1 Minute	5	6.20	3.114	–0.635	8	0.543
	After 2 Minutes	5	8.00	5.523			

** indicated significant difference at $p \leq 0.05$.*

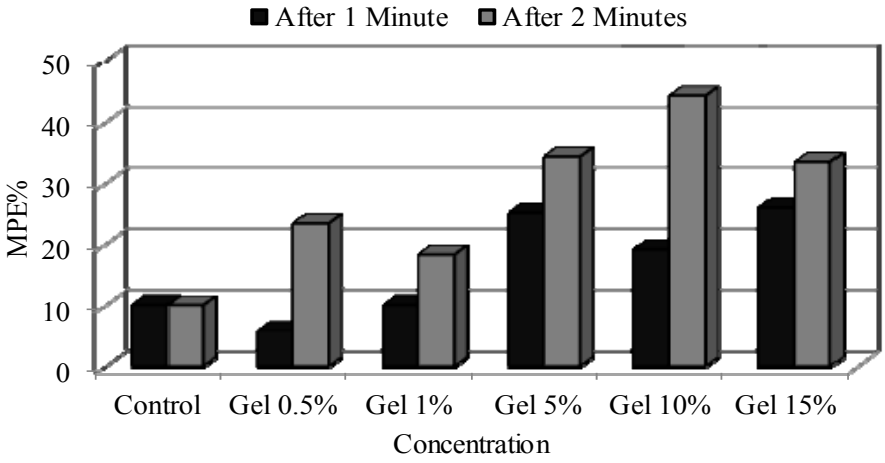

Figure 5. Effect of ketamine gel (0.5 , 1 , 5 , 10 , 15)% on antinociceptive maximum pain effect (MPE) in hot plate test after 1and 2 min.

DISCUSSION

ketamine is classified as an NMDA receptor antagonist [9] . Our findings suggest that topical application of Gel containing KET within the range of concentration from 0. 5% to 15% is an additional approach to attenuate painful stimulated by thermal (Hot-plate) in mice. The analgesic activity depended on concentration of ketamine. This result agreement with previous study suggested that topical application ketamine demonstrate efficacy in neuropathic and nociceptive pain [16,17] . The analgesic efficiency of ketamine gel it's may be attributed to it's action on NMDA receptors[7].

Many studies identified several glutamate receptors, such as NMDA, amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and kainate with action on unmyelinated, myelinated, and postganglionic sympathetic axons. It was suggested that these peripherally distributed receptors play a role in the transmission of sensory signals to the central nervous system. [18,19]. Glutamate is the primary excitatory neurotransmitter of central nervous system and is normally released by pain-signaling afferent neurons as they synapse on central pain pathways in the spinal cord. The persistence release of glutamate, due to peripheral injury or inflammation, leads to the activation of N-methyl-D-aspartate (NMDA) receptors. This process of activation has been shown to play a crucial in mediating the phenomena of pain [20] . This activation can be prevented mitigated by agents that block the effects of glutamate at NMDA receptor [21, 22] . These discoveries have promoted attempted to use NMDA receptor antagonist in the treatment of neurogenic and other, often difficult to control, pain state [23]. NMDA receptor antagonism effects analgesia by preventing central sensitization in dorsal horn neurons; in other words, ketamine's actions interfere with pain transmission in the spinal cord [24] .

Another possible explanation of the analgesic activity of ketamine gel, its interacts with sigma and opioid μ receptors [25-27] . Ketamine also inhibits nitric oxide synthase, inhibiting production of nitric oxide, a neurotransmitter involved in pain perception, and hence further contributing to analgesia [28] . another mechanism of analgesic action of ketamine due to blocks voltage-sensitive calcium channels and depresses sodium channels, attenuating hyperalgesia; Finally ketamine alters cholinergic neurotransmission, which is implicated in pain mechanisms; and it acts as a noradrenergic and serotonergic uptake inhibitor, which are involved in descending antinociceptive pathways [29].

CONCLUSION

Although only a small number of topical agents are available for use in peripheral and local conditions, the obtained results demonstrate that the topical ketamine may provide clinicians with anew option in the battle against pain

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پوخته

دەرئێخستنا کیتامینێ توز و خواندنا کارتێکرنا وی وەك ئێش سڤککەر ل سەر مشکا ب رێکا ب کارئینانا پلێتا گەرم

رێکێن ڤەکولینی:دڤێ ڤەکولینێ دا توزا کیتامین هاتە دەرئێخستن ژ (محلول)ێ کیتامین هایدروکلورایدێ. پاقژیا ڤێ توزێ هاتە تاقیکرن ب رێکا ئامیرێ تیشکێن دبن سوری دا و د سەر موری دا. جێلا کیتامین هاتە دروستکرن ژ توزا کیتامین ب چەند ڕێژەیەکا (0.5, 1, 10, 15) ب مەرەما هەلسەنگاندنا کارتێکرنا جێلا کیتامینی وەك سڤککەرێ ئێشێ.

ئەنجام: ئەنجامێن ڤەکولینێ دیار کر کو توزا کیتامین یا دەرئێخستی یا پاقژە و ئەف چەندە هاتە دوپات کرن ب رێکا ئامیرێ تیشکێن دبن سوری دا و د سەر موری دا و هەر وەسا دیار بوو کو جیلا کیتامینی ب ڕێژێن ل سەری دیار کری رولەکێ باش هەبوو بو سڤککرنا ئێش و ئازارا ل دەف مشکا پشتی بورینا (4.4+-2) (2.1 -8.2+) (10.6+-5.2) (8+-2.1) (5.6+-2.5) چرکە ل دویف ئێك پشتی بورینا دوو خولەکان ل دانانا جیلی ب رێکا ب کارئینانا پلێتا گەرم بەرامبەر گروپێ کونترولێ (2.4+-2). و هەروەسا ڕێژا سەدی یا سڤککرنا ئێشێ زیدە بوو ژ (9.9٪) ل گروپێ کونترولێ هەتا ((23.3) (8.3) (34.2) (44.2) (33.3) ٪) و لدویف ڕێژا هلامیا کیتامینی (0.5, 1, 10, 15) پشتی دوو خولەکا ژ بکارئینانێ.

دەرئەنجام: دەرئەنجامێ ڤێ ڤەکولینێ ئەوە کو چێدبیت توزا کیتامینێ بهێتە دەرئینان ژ (محلول)ێ کیتامینا هایدروکلورایدی و بهێتە ب کارئینان وەك جیل بو سڤککرنا ئێش و ئازاران.

الخلاصة

استخلاص مسحوق الكيتامين ودراسة تأثيره المسكن كهلام في الفئران باستخدام اختبار الصفيحة الساخنة

طريقة البحث:تم في هذه الدراسة استخلاص مسحوق الكيتامين من محلول الكيتامين هايدروكلورايد عن طريق ترسيبه. بعد ذلك تم اختبار نقاوة هذا المسحوق عن طريق جهاز مطياف الاشعة تحت الحمراء و فوق البنفسجية. ومن مسحوق الكيتامين تم تحضير هلام الكيتامين بعدة تراكيز (0.5 , 1 , 5 , 10 , 15)٪ لتقييم تأثيره جيل الكيتامين كمضاد للألم.

النتائج: أظهرت النتائج إن مسحوق الكيتامين المستخلص نقي حيث تم تأكيد هذه النتيجة بجهاز مطياف الأشعة تحت الحمراء و فوق البنفسجية كما وأعطى هلام الكيتامين بتركيز (0.5, 1 , 5 , 10, 15)٪ تسكين جيد من الألم في الفئران (5.2±10.6)(2.1±8) (2.5±5.6) (2±4.4) (2.1±8.2) ثانية على التوالي بعد دقيقتين من وضع الهلام باستخدام اختبار الصفيحة الساخنة بالمقارنة مع مجموعة السيطرة (2±2.4). ولقد ازدادت النسبة المؤية للتسكين من الالم من (9.9) ٪ في مجموعة السيطرة الى (23.3)(18.3)(34.2)(44.2) (33.3) ٪ على التوالي وحسب تركيز هلام الكيتامين (0.5 , 1 , 5 , 10 , 15)٪ على التوالي بعد دقيقتين من استخدامه.

الخلاصة: لقد توصلت الدراسة الحالية إلى إمكانية استخلاص مسحوق الكيتامين من محلول الكيتامين هايدروكلورايد وإمكانية استخدام استخدام هلام الكيتامين لإزالة الألم باستعماله موضعيا.

VP16-LXRB ACT AS BOTH ANTIPROLIFERATIVE AND LIPOGENIC FACTORS IN MCF-7 BREAST CANCER CELL LINE

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ABSTRACT

Background and Objectives: Liver X receptors (LXRs) belong to the nuclear receptor superfamily of ligand-dependent transcription factors, LXRs are activated by endogenous Oxysterols, metabolites of cholesterol, and therefore act as intracellular sensors of this lipid. The Oxysterol receptors (LXRα and LXRβ) regulate cholesterol and lipid biosynthesis; particularly reverse cholesterol transport (RCT). One complicating factor in studies utilizing synthetic LXR agonists in the potential for pharmacologic and receptor-independent effects. Here, we show that an LXR gain-of-function system that does not depend on the addition of exogenous ligand.
Methods: We transfected the cells of MCF-7 cell line by a specific recombinant plasmid of VP16-LXRα and VP16-LXRβ instead of addition of exogenous synthetic agonists (such as GW3965 or T0901317), Analysis of gene expression in MCF-7 cells treated with recombinant plasmid confirmed by qPCR and Western Blot Analysis.
Results: These cells exhibit increased LXR signaling. Analysis of gene expression on mRNA and protein levels in transfected MCF-7 cells with VP16-LXR confirmed that the ability of LXR to drive expression of genes involved in cholesterol efflux and fatty acid synthesis (SCD1, SREBP1c, ABCA1 and ABCG1). Furthermore, we demonstrated that significantly reduced the proliferation in MCF-7 human breast cancer cell line, LXR suppressed mRNA and/or protein expression of SKP2, while it increased the expression of P53 at the protein level.
Conclusions: The net results of this study showed that the transcriptional activation of VP16-LXRβ act as both antiproliferative and regulation of key lipogenic LXR target genes in MCF-7 human breast cancer cell line.

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Key words: VP16-LXRβ, antiproliferative, lipogenic factor and MCF-7 cell lines.

Liver X receptors (LXRs) are ligand-activated transcriptional factors that belong to the nuclear receptor superfamily. LXRs are important regulators of cholesterol, fatty acid, and glucose homeostasis [4]. There are two LXR isoforms. LXRα (NR1H3) expression is most abundant in liver, kidney, intestine, fat tissue, macrophages, lung, and spleen [4], it was initially isolated from a rat liver cDNA library as a novel orphan nuclear receptor, i.e. receptors with no known physiological ligands [1], while LXRβ (NR1H2) is ubiquitously expressed [4].
The herpes simplex virus virion protein, VP16 is a potent transcriptional activator. As a transcriptional regulatory protein, it contains two functional domains. The aminoterminal portion of the protein, in association with host cellular proteins [26,27]. The transcriptional enhancement activity resides in the carboxyl-terminal 78 amino acids [6]. This domain can strongly activate transcription in various systems when attached to the DNA-binding domain of a heterologous protein [20].
Human LXRα and LXRβ share almost 80% amino acid identity in their DNA-binding domain and ligand-binding domain. The LXR paralogs are highly conserved between rodents and humans. Human LXRα and rat LXRα show close to 100% homology in amino acid sequence in their DNA-binding domain and ligand-binding domain [15]. With the discovery of

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oxysterols [11] as endogenous ligands for LXRs, these receptors were included in the group of 'adopted' nuclear receptors, i.e. receptors where a physiological ligand has been identified subsequent to the identification of the receptor [28]. As regulators of metabolism, LXRs have been considered as potential drug targets by the pharmaceutical industry, and synthetic LXR ligands have been developed, which are widely used as tools in biomedical research. Synthetic LXR ligands include T0901317 [21] and GW3965 [5].

LXRs regulate cholesterol transport in the liver and macrophages and under normal circumstances prevent atherosclerosis [19]. Synthetic LXR ligands potentially could be used to treat diseases such as atherosclerosis [23], but their lipogenic effect in the liver causes hypertriglyceridemia, an undesirable side effect [17].

A wide variety of cancers show increased lipogenesis and expression of lipogenic enzymes including SREBP1c, FAS and ACC [22]. Interestingly, recent work has identified LXRs as anti-proliferative factors suppressing growth of both normal and cancer cells [25] Activation of LXRs suppresses proliferation [3,8].

LXR agonists appeared to cause G1 cell cycle arrest in prostate cancer cells by reducing expression of S-phase kinase-associated protein 2 (Skp2), which resulting in the accumulation of cell cycle inhibitor p27Kip [8]. In MCF-7 breast cancer cells, treatment with synthetic LXR agonists T0901317 and GW3965 suppressed the mRNA and protein expression of Skp2, cyclin A2, cyclin D1 and estrogen receptor (ER) α, while it increased the protein expression of p53 and decreased the phosphorylation of serine 780 and 795 of retinoblastoma (Rb) [24]. Reduced phosphorylation at these two sites is related to an active form of the Retinoblastoma (Rb) protein that binds E2F and inhibits cell cycle progression. T0901317 is a very potent LXR agonist, its effective concentration to activate LXRα is 20 nM [21]. The aim of this study is to show that VP16-LXRs are anti-proliferative factors in MCF-7 human breast cancer cell line.

METHODS

Cell culture

Human breast adenocarcinoma cell line, MCF-7, was cultured in DMEM medium (Invitrogen, Carlsbad, CA) containing 5.6 mM glucose, 1 mM sodium pyruvate, 4 mM L-glutamine, 25 mM HEPES and 2 to 10% fetal bovine serum (FBS, Saveen Werner, Malmö, Sweden). The cell culture media were supplemented with 100 Units/ml penicillin and 100 µg/ml streptomycin (Invitrogen) in a humidified 37ºC incubator with 5% CO2. This cell line was obtained from Vedin L. and Hassan T., Ph.D. students, Bioscience and Nutrition Department, Karolinska Institutet, Stockholm, Sweden

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Transfection of the cells

A day before transfection the cells plated into 6 cm dish so that they were 80-90% confluent for the day of transfection. In this experiment 2µg for each Empty, VP16-LXRα and VP16-LXRβ plasmids were diluted in free serum and antibiotic media (Media without Fetal Bovine Serum {FBS} as well as without Penicillin and Streptomycin {PEST}) according to the special experimental design as follow:

In the other tube dilute 6-10 µl (10µl) of Lipofectamine into 290 µl DMEM without FBS/well were used and incubated for 5 minutes at room temperature (RT), then diluted plasmid (Table 1) and diluted Lipofectamine (Table 2) were combined and mixed gently and incubated for 15-45 minutes (30 min.) at RT to allow DNA-liposome complex to form. During this period (30 min.) the media of the pales were exchanged with 3 ml of free serum media (Free of FBS and PEST). Then 600 µl of the mixture were added into each well containing 1.4ml (1400 µl) media free serum. The plates were incubated for 4-6

hrs. (5 hr.) at 37 °C. Following incubation, media were replaced with 2 ml (2000 µl) of treated media with 1% FBS, then incubated O/N. A day later both RNA and Protein of the cells were extract for further examination.

Table 1. Preparation of the desired plasmid (Empty, VP16-LXRα and VP16-LXRβ) concentration and volume.

Total Vol./Tube (µl)	Free serum media Vol. (µl)	Plasmid Desired Vol. (µl)	Plasmid Conc. (µg/µl)	Plasmids	Tube No.
300 µl	299 µl	1µl	2 µg/µl	Empty	1.
300 µl	297.1 µl	2.9 µl	0.7 µg/µl	VP16-LXRα	2.
300 µl	297.8 µl	2.2 µl	0.9 µg/µl	VP16-LXRβ	3.

Table 2. Preparation of the desired Lipofectamine concentration and volume.

Total Vol./Tube (µl)	Free serum media Vol. (µl)	Desired Vol. (µl)	Lipofectamine	Tube No.
300 µl	290 µl	10µl	Lipofectamine	1.
300 µl	290 µl	10 µl	Lipofectamine	2.
300 µl	290 µl	10 µl	Lipofectamine	3.

Quantitative PCR (qPCR)

Total RNA was isolated using the E.Z.N.A. Total RNA kit I (Omega Bio-Tek Inc., Norcross, GA), 500 ng RNA was reverse-transcribed using the SuperScript II reverse transcriptase kit (Invitrogen). Real time qPCR was performed using the SYBR Green technology using the Power SYBR mastermix (Applied Biosystems) and amplified in an 7500 fast real time PCR system (Applied Biosystems). Primers were designed using Primer Express software; primer sequences are available on request. We calculated relative changes by the comparative CT-method using 18S and/or 36B4 as the reference genes.

Western blot

For protein expression levels in MCF7 cells, 5 x 105 cells were plated in 6 well plates. Medium was replaced after 16h and cells treated with Empty, VP16-LXRα and VP16-LXRβ plasmids for 72h. Cells were harvested directly in 1xSDS protein loading buffer and boiled for 5 min. The proteins were separated on 8 to 12.5% SDS-polyacrylamide gels using standard procedures. The antibodies used were against Skp2 p45 (H-435), p53 (DO-1) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), SCD1 (CD.E10) (GeneTex, Inc., San Antonio, TX), SREBP-1c (BD Bioscience, BD Pharmingen, USA), ABCG1 (abcam Inc, US, UK), ABCA1 (abcam INc, US, UK), α-Tubulin (Cell signaling technology, Inc.) and β-actin (Sigma-Aldrich).

RESULTS

To further our understanding of the function of LXR we used a model of VP16-LXRs overexpression. A transgenic expression vector was engineered to generate an N-terminal-VP16 activation domain-LXR -fusion protein. The expression of LXRs after the addition of the VP16 transcriptional activation domain was studied to find its functions on both mRNA and protein levels. Real-time quantitative PCR analysis confirmed the expression of the VP16-LXRs. In addition, Western blot analysis verified that the VP16-LXRs were expressed selectively in MCF-7 cell lines.

Many authors have previously shown that LXRs contribute to the regulation of genes involved in lipid metabolism. Consistent with prior studies employing synthetic LXR ligands, VP16-LXRs exhibited increased expression of the LXR target genes against an internal control (Fig. 1A, 1B), i.e. Stearoyl-CoA desaturase-1 (SCD1) (Fig. 1C), sterol-regulatory element binding protein (SREBP)-1c (Fig. 1D), ATP Binding Cassette transporter isoforms A1 (ABCA1) (Fig. 1E), and ABCG1 (Fig. 1F).

This observation confirms that the LXR - VP16 fusion protein functions as a constitutively active receptor for the key lipogenic genes. Contrary to the proliferation genes, i.e. S-phase kinase-associated protein 2 (SKP2) (Fig. 1G), which was significantly decreased on both mRNA and protein levels, however, we have not observed consistent alterations in the tumor protein 53 (p53) in the same experiment (Fig. 1H).

LXRs inhibit proliferation in human breast cancer cells. MCF-7 breast cancer cell line was established as a model system to analyses LXR responses. MCF-7, expressed both LXRα and LXRß and showed induced expression of known LXR target genes including ABCA1 and SREBP1c, ABCG1 and SCD1 upon activation of LXRs with the GW3965 LXRα and LXRß selective agonist. Hence, we conclude that LXRs are functional in these cells and can be activated by an agonist. We analyzed the effect of VP16-LXRs on proliferation of the cells in the S-phase of the cell cycle by using qPCR with SKP2 primer and western blot with SKP2 antibody.

As we mentioned that LXRs regulate expression of cell cycle genes and lipogenic genes in MCF7 cells. We performed mRNA and protein expression profiles in an effort to elucidate the detailed molecular mechanisms underlying the anti-proliferative effect of LXRs. Interestingly, the mRNA induction of lipogenic target genes including SREBP1c, SCD1, ABCA1 and ABCG1 peaked at 2µg of recombinant VP16-LXRß plasmid concentration. In contrast, expression of gene involved in cell-cycle progression such as Skp2 was suppressed by the same concentration of VP16-LXRß as repressed proliferation. These mRNA expression profiles of VP16-LXRß were not paralleled at the protein level for SCD1 and ABCG1, but they were paralleled for SREBP-1c and Skp2. while the internal control GAPDH and α-Tubulin remained unchanged, whereas p53 protein levels were induced by VP16-LXRß (Figure 2).

These results of the present study confirm that lipid metabolism is involved in cell cycle regulatory events, but that the anti-proliferative effect of LXRs is independent of their role in lipogenesis.

ABCG1

GAPDH

DISCUSSION

The identification of high-affinity synthetic agonists for nuclear receptors has enabled many studies on the biological functions of these nuclear receptors. The availability of antagonists has also been important to demonstrate that a given effect is indeed mediated by a nuclear receptor. However, the development of pure agonists and antagonists are only rarely the goal in therapeutic strategies based on nuclear receptors as drug targets, when attempting to improve the pharmacological profile of new molecules. In most cases, partial and selective modulators (agonists or antagonists) are desired, which activate or block the receptor in a tissue specific manner and/or present target-gene specificity [7].

In addition to endogenous ligands, a number of synthetic LXR ligands have been developed. Most of them are dual

agonists, activating both LXRα and LXRβ, and present both the favorable effects on cholesterol metabolism and the unfavorable effects on fatty acid metabolism. Treating mice deficient in either LXRα, LXRβ or both, with an agonist displaying equal potency for both isoforms or an agonist selective for LXRα, demonstrated that specific activation of LXRα or LXRβ yields distinctive lipid outcomes in vivo [16]. Most importantly, this study lends further support to the hypothesis that a VP16-LXRβ would increase the expression of ABCA1 in MCF-7 (stimulating therefore cholesterol efflux). This particular interest for LXRβ has been supported by another study in which selective activation of this isotype by treatment of LXRα-deficient mice with a dual agonist results in a strongly decreased expression of hepatic genes concomitant with an increased expression of both the ABCA1 and SREBP1c genes in peripheral tissues (kidney and duodenum), causing plasma HDL increases without hypertriglyceridemia [18].

The most important finding of this work is that VP16 selectively activated with LXRβ but not with LXRα on mRNA level. This result may explain some of the functional differences between the two LXR isotypes. LXRα and LXRβ bind the same ligands and bind to the same promoter DNA sequences. Therefore, the functional dominance of LXRα is likely regulated by differences between the LXR isotypes in yet unidentified mechanisms. One of these mechanisms may be related to the inhibitory effect on the ligand-induced transcriptional activity of LXRβ. It is likely that, selective protein-protein interactions regulate the functions of LXRα and LXRβ [13]. In addition to the reported anti-proliferative effects of LXRs in human cancer cell lines, outlined in the introduction, activation of LXRs also reduced proliferation and caused growth arrest in vascular smooth muscle cells [2] .In keratinocytes, LXRs induces epidermal differentiation and inhibits hyperproliferative epidermis in vivo [14].

Recently, an indirect antiproliferative role of LXRs was documented where activation of LXR induced hepatic expression of the estrogen deactivation enzyme, estrogen-sulfotransferase, which led to inhibition of breast cancer growth in a nude mouse model of tumorigenicity [9]. In this study we provide evidence for a strong anti-proliferative effect of VP16-LXRs in human breast cancer cell lines. Signaling lipids including fatty acids participate in control of cellular processes such as metabolism and cell proliferation. LXRα and LXRβ are important sensors of cholesterol and lipid metabolism. We observed less proliferation of MCF7 cells when transfected with VP16-LXRβ. Interestingly, LXRs might have a dual role in this respect. First, LXRs are anti-proliferative in the breast cancer cells. Second, LXR-induced expression of lipogenic genes peaked at lower agonist (VP16) concentration than needed for the anti-proliferative effect of LXR. Hence, regulation of lipid production by LXRs seems to be mechanistically different from that of the anti-proliferative effect of LXRs.

Here we showed that a strong anti-proliferative effect of LXRs is dependent on activation of LXRs with VP16, which reduced expression of the mRNA and/or protein level of Skp2. LXRs induced expression of p53 at the protein level which was associated with the active form of p53.

These observations strongly suggest that VP16-LXRs act upon several molecular targets regulating cell cycle which is in line with our observations that there is also a small, but significant, anti-proliferative effect in MCF-7 cells. Nuclear receptors in general and LXRs in particular are well suited targets for drug development as they are transcription factors easily activated/deactivated by small compounds that can penetrate the cell membrane and modulate receptor activity in vivo. LXRs

are interesting drug targets for pharmacological intervention of various metabolic disorders. The present and other recent reports establishing LXRs as regulators of cell growth indicate that LXR signaling may also be a potential target for anticancer drugs. The nuclear receptors LXRα and LXRβ have been shown to play important roles in both inflammatory and metabolic pathways. Prior studies have relied on synthetic LXR ligands and in vitro overexpression systems [10].

Here, we describe a novel in vitro overexpression model in which VP16-LXRα and VP16-LXRβ are constitutively expressed in MCF-7 cells, in which the VP16-LXRs were positively regulated known target genes. Furthermore, in this overexpression system model we provided additional in vitro evidence for metabolic and antiproliferation roles of LXRs in relation to recombinant VP16 plasmid.

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پوختە

MCF-7کاردەکات وەك هەردوو هۆکاری دژەتۆرمبوون و چەوری لە خانەی شێرپەنجەی مەمك

لەجۆری VP16-LXRβ

پێشینەو ئامانجەکان: وەرگرەکانی جگەر لەجۆری X دەگەڕێنەوە بۆ سەرووی خێزانی ناوکە وەرگرەکانی پشت بەست بە بەستەرە هۆکارەکانی کۆپیکردن, LXR ەکان چالاك دەکرێن بەهۆی ئۆکسی ستیرۆڵی ناوەکی, کە بریتی یە لە بەرهەمەزیندەگی یەکانی کۆلیتیرۆڵ, بۆبە وەکو هەستپێکی ناو خانە کاردەکات بۆ ئەو چەوریە. وەرگرەکانی ئۆکسی ستیرۆڵ (هەردوو جۆری ئەلفاو بیتا لە وەرگرەکانی جگەر لەجۆری X) کۆلیستیرۆڵ و زیندە دروستبوونی صەوری ڕێکدەخەن, بە تایبەتی گواستنەوەی کۆلیسترۆڵی گەڕاوە. یەکێك لە هۆکارە ئالۆزەکانی ئەو لێکۆڵینەوانە ئەوەیە کە بەستەرە هۆکاری دەستکرد بەکاردەهێنن بۆ وەرگرەکانی جگەر لەجۆری X لە لایەنی دەرمانسازی و کاریگەریە پشت نەبەستووەکانی وەرگرەکان. لێرەدا ئێمە ئەوە پیشان دەدەین کە وەرگرەکانی جگەر لەجۆری X فەرمانی خۆیان جێ بەجێ دەکەن بەبێ پشت بەستن بە زیادکردنی بەستەری دەرەکی خانەکان.

ڕێگاکان: خانەکانی MCF-7 مان تووشکرد بە پلازمیدێکی تایبەتی دووبارە هەڵچنراو بە VP16-LXRa وە VP16-LXRb لەجیاتی زیادکردنی بەستەرەهۆکاری دەستکردی دەرەکی وەکو GW3965 وە T0901317, شیکارکردنی دەربڕینی بۆهێلەکان لە خانەکانی MCF-7 ی مامەڵەپێکراو بەو پلازمیدە دووبارەهەڵچنراوە جەختی لەسەرکراوە بە شیکاریەکانی qPCR و بلۆتی وێسترن.

ئەنجامەکان: ئەو خانانە زیادبوونی نیشانەپەیوەندیەکانی وەرگری جگەر لەجۆری X پیشاندەدەن. شیکارکردنی دەربڕینەبۆهێلەکان لەسەر هەردوو ئاستی mRNA (RNA نێرەر) و پرۆتین لە خانە تووشکراوەکانی MCF-7 بە پلازمیدی VP16ی بەستراو بە وەرگری جگەر لەجۆری X جەخت لەسەر توانای وەرگری جگەر لەجۆری X دەکەنەوە بۆ بەکارخستنی دەربڕینەبۆهێلەکانی پەیوەست بە کرداری دروست بوونی ترشە چەوریەکان و دەرپەڕاندنی کۆلیستیرۆڵ (SCD1, SREBP1c, ABCA1 وەABCG1). زیاتر لەوە ئێمە ئەوەمان نیشانداوە کە بەشێوەیەکی واتایی زیادبوونی خانەکانی شێرپەنجەی مەمك لەجۆری MCF-7 لە دەرەوەی لەشی زیندوو واتە لە تاقیگە کەمیکرد, وەرگرەکانی جگەر لەجۆری X دەبێتەهۆی کپکردنی دەربڕینە بۆهێلیەکان لە ئاستی mRNA وە یان پرۆتینی SKP2, بەڵام بووەهۆی زیادبوونی دەربڕینەکانی بۆهێلی p53 لەسەر ئاستی پرۆتینی.

دەرئەنجامەکان: ئەنجامە بێگەردەکانی ئەم لێکۆڵینەوەیە پیشانیاندا کە چالاکی کۆپی کردنیVP16-LXRβ کاردەکات وەك کەم بوونەوەی خانەکان و ڕێکخستنی بۆهێلە ئامانجەکانی وەرگرەکانی جگەر لەجۆری X پەیوەست بە کردارەکانی دروست بوونی چەوری لە خانە شێرپەنجەیی یەکانی مەمك لەجۆری MCF-7 .

الخلاصة

عمل VP16–LXR بمثابة أنتيبروليفرتيف واكتناز الدهون على حد السواء على خط MCF–7 خلايا سرطان الثدي

الخلفية والأهداف مستقبلات الكبد X (LXRs) تنتمي إلى فصيلة المستقبلات النووية التي تعتمد على عوامل النسخ ، يتم تنشيط مستقبلات الكبد الاوكسيستيرول الذاتية، ونواتج الايضي للكوليستيرول ، وبالتالي تكون بمثابة أجهزة الاستشعار داخل الخلايا الدهنية. مستقبلات الاوكسيستيرول ومستقبلات الكبد الفا و البيتا تنظم الكولسترول العمليات الحيوية للدهون و خاصة نقل الكوليسترول المعاكس ومن العوامل المعقدة في دراسة استخدام مستقبلات الكبد الاصطناعية في إلامكانيات الصيدلانية وتاثير على العوامل المستقلة .

طريقة البحث : نقلنا خط الخلايا MCF-7 بخلية البلازميد المؤتلف والمحددة من VP16 LXRα-VP16 و–LXRβ بدلا من إضافة منبهات الخارجية الاصطناعية (مثل GW3965 أو T0901317)، وتحليل التعبير الجيني في الخلايا MCF-7 المتعامل و المؤكد ببلازميد qPCR و الويسترن بلوت.

النتائج: اظهرت هذه الخلايا زيادة إشارات LXR. أكد تحليل التعبير الجيني على مستويات مرنا والبروتين في الخلايا بالنقل–MCF–7 مع LXR VP16 أن قدرة LXR لدفع التعبير عن جينات لها دور في هروب رأس المال والكولسترول الدهنية تركيب الحامض (SCD1، SREBP1c، وABCG1 ABCA1). قمعت LXR وعلاوة على ذلك، فإننا أظهرت قبعة إلى خفض كبير في الانتشار في خط MCF-7 الثدي الخلايا السرطانية البشرية، مرنا و / أو التعبير البروتين من SKP2، في حين أنها زادت من التعبير عن P53 على مستوى البروتين.

الاستنتاجات: أظهرت النتائج صافي من هذه الدراسة أن تفعيل الترانسكربتي من VP16-LXRβ الفعل على حد سواء antiproliterative وتنظيم الجينات المستهدفة الرئيسية اكتناز الشحم في LXR-7 MCF خط الثدي الخلايا السرطانية البشرية.

VALIDITY OF TWO-DIMENSIONAL ECHOCARDIOGRAPHY IN THE DIAGNOSIS OF CORONARY ARTERY DISEASE

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ABSTRACT

Background: Coronary artery disease is the leading cause of morbidity and mortality both, in the developed and the developing world. Oftentimes further diagnostic tests are needed for the diagnosis of coronary disease in addition to history and physical exam. Few studies have been performed to compare two-dimensional resting echocardiographic findings directly with coronary angiography. Aim of Study: This study was conducted to estimate the validity of two-dimensional echocardiography in the diagnosis of coronary artery disease.
Methods: A cross-sectional study was conducted at Azadi General Teaching Hospital in Duhok City. Data were collected from January 20, 2010 to May 8, 2010. A consecutive sampling procedure was used to enroll a total of 300 adult patients (164 men) who had undergone two-dimensional echocardiography and were referred for coronary angiography.
Results: Most of the patients (86%) were 40 to 69 years old. The overall two-dimensional echocardiographic sensitivity, specificity, and accuracy were 58%, 88%, and 68%, respectively. The negative predictive value of two-dimensional echocardiography was higher in women than in men (66% vs. 31%, $p < 0.01$). The more coronary arteries are diseased the more echocardio-graphy corresponds to the angiographic results. Out of the six echocardio-graphic findings, wall motion abnormality and left ventricular dysfunction showed the highest association with coronary artery disease ($p < 0.001$).
Conclusions: The overall validity of two-dimensional echocardiography in the diagnosis of significant coronary artery disease is limited. With a better specificity than sensitivity it is useful for ruling in coronary artery disease but should not be used to rule out significant disease.

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Key words: Coronary artery, Echocardiography, Coronary angiography.

Cardiovascular diseases (CVD) are among the leading causes of mortality and morbidity. The major underlying cause of CVD is the progression of coronary artery disease (CAD) or narrowing of the coronary arteries due to atherosclerosis,[1] presenting asymptomatically or as angina, acute coronary syndrome, myocardial infarction, arrhythmias, or chronic heart failure.[2]
The diagnosis of CAD is often difficult to establish, especially in those patients who are asymptomatic for the disease, and in those who have atypical chest pain associated with a normal resting electrocardiogram (ECG). Early diagnosis is mandatory as it may benefit the patient to be treated properly medically, or through angiographic interventions.[3]

Coronary angiography remains the standard for identifying the presence or absence of arterial narrowing related to atherosclerotic CAD [4,5] It is limited, however, by its invasiveness and is associated with risks of serious complications, thus there is a growing interest in non-invasive technologies to diagnose obstructive CAD.[6] One non-invasive diagnostic modality is two-dimensional echocardiography, which is well accepted for the evaluation of myocardial function in patients with known or suspected CAD.[7] Although association of CAD and some isolated echo findings have been examined, few studies have performed a direct comparison of different echo features in predicting CAD as compared to the gold

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standard, coronary angiography.[8]

Due to a lack of studies about the validity of non-stress 2D-Echo in the diagnosis of CAD in Duhok governorate, and with the recent establishment of the Duhok Cardiac Center, in 2008, this study was conducted in Azadi Teaching Hospital to assess the validity of 2D-Echo in the diagnosis of CAD.

METHODS

This study was conducted in the Cardiac Angiography Unit at Duhok Cardiac Center in Azadi General Teaching Hospital in Duhok City.

The study is a cross-sectional study. The investigator interviewed the eligible patients (age 18 years or above, having a previous 2D-Echo report, being prepared for diagnostic coronary angiography), and exclude patients with established diagnosis of CAD or prior coronary angiography, Patient prepared for therapeutic coronary, Decomponsated heart failure, Congenital heart disease, Valvular heart disease.

A consecutive sampling procedure was used to include 300 patients (164 men and 136 women) who underwent 2D-Echo and the data were collected from January 20, 2010 to May 8,2010.

Statistical Analysis All the data was entered into Microsoft Office Excel 2003 and transferred to SPSS 15.0 for statistical analysis. The chi square ($\chi 2$) and p-values were obtained after entering the data into two-by-two tables in OpenEpi 2.3, using the uncorrected chi square and a 2-tailed p-value. For two-by-two tables that did not meet Cochran's criteria for accepting the chi square (no more than 20% of cells have expected < 5 and no cell has an expected value < 1), the Fisher exact test was used .The multinomial logistic regression analysis function in SPSS 15.0 was used to assess the correlation between the echocardiographic findings, and the angiographic results.

RESULTS

The study sample included 300 patients comprising 164 men and 136 women. Most of the patients (37%) were in the age group 50-59 years. There were more men than women in the age groups from 40 to 59 years as shown in (Figure1).

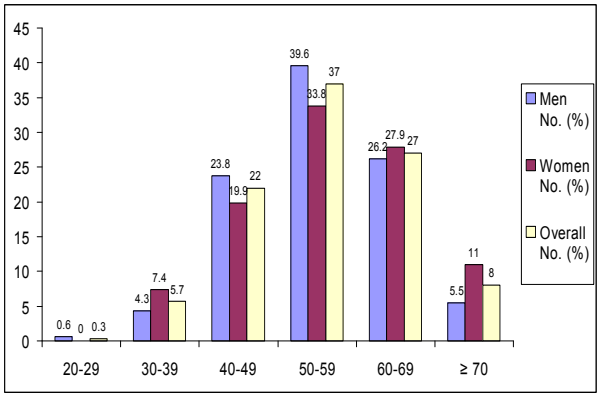

Figure 1. Study Population by Age and Gender

The women were slightly older than the men (not statistically significant). The most common echocardiographic abnormality was wall motion abnormality (WMA) (41.7%) which show also gender differences (53.7% men vs. 27.2% women) followed by left ventricular diastolic(LVD) dysfunction. The coronary angiographic results show that more than 70% of the study population suffer from coronary artery disease. In both genders, there were about twice as many in whom significant CAD was diagnosed as compared to severe CAD as shown in (Table1).

(Table 2) represents a summary of the validity measures of both men and women, showing the differences and similarities of the echocardiographic detection in terms of sensitivity, specificity, accuracy PPV(Positive predictive value), and NPV(Negative predictive value) .

A summary statistics comparing the differences in validity measures in (Table 2) by gender in terms of p-values, all comparisons proved statistically insignificant apart from the NPV which

Table 1. Echocardiographic and Angiographic Characteristics of the Study Sample by Gender

Characteristics	Men		Women		Overall	
Number	164		136		300	
Age ± SD	54.2	±6.6	55.6	±10.3	54.8	±8.6
2D-Echo Findings						
Wallmotion Abnormalities	88	(53.7)	37	(27.2)	125	(41.7)
Dilatation of LV	37	(22.6)	14	(10.3)	51	(17.0)
EF < 50%	20	(12.2)	9	(6.6)	29	(9.7)
LV D Dysfunction	53	(32.3)	46	(33.8)	99	(33.0)
Mitral Valve Regurgitation	32	(19.5)	16	(11.8)	48	(16.0)
Thinning of Septum	10	(6.1)	6	(4.4)	16	(5.3)
Coronary Disease						
No CAD	21	(12.8)	57	(41.9)	78	(26.0)
Non-Significant CAD	8	(4.9)	12	(8.8)	20	(6.7)
Significant CAD	91	(55.5)	46	(33.8)	137	(45.7)
Severe CAD	44	(26.8)	21	(15.4)	65	(21.7)

Table 2. The Estimated Validity Measures by Gender

Measures	Men and Women	Men	Women
Sensitivity	58.42%	62.22%	50.75%
Specificity	87.76%	79.31%	91.30%
Accuracy	68.00%	65.24%	71.32%
PPV	90.77%	93.33%	85.00%
NPV	50.59%	31.08%	65.63%

reflects significant differences between the three categorized groups as shown in (Table 3). (Figure 2) comparing the six echocardiographic findings, it shows that wall motion abnormalities were highly associated with CAD and to a lesser degree left ventricular dysfunction, an ejection fraction of less than 50%, and dilatation of the left ventricle (LVD). Still significantly associated was mitral valve regurgitation while thinning of the septum barely missed statistical significance.

Table 3. Statistical Analysis* of the Estimated Measures

Measures	All vs. Men	All vs. Women	Men vs. Women
Sensitivity	**0.51**	**0.30**	**0.12**
Specificity	**0.30**	**0.46**	**0.10**
Accuracy	**0.55**	**0.48**	**0.30**
PPV	**0.50**	**0.30**	**0.13**
NPV	**< 0.01**	**0.01**	**< 0.01**

** Based on z - test*

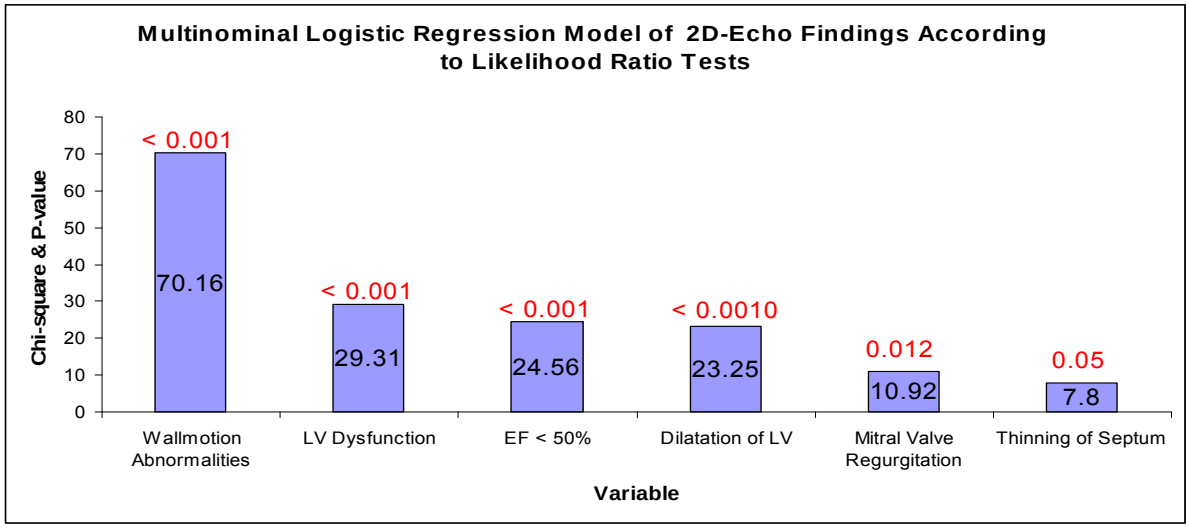

Figure 2.Multinominal Logistic Regression Model of 2D-Echo Findings According to Likelihood Ratio Tests

In (Table 4) the Validity of 2D-Echocardiography by the Extent of Coronary Vessel Involvement as it compare the echocardiographic results with the extent of coronary vessels involvement, the numbers of false positive and true negative echocardiographies remain the same, thus resulting in a specificity of almost 88%. It shows that 2D-Echo has a sensitivity of almost 50% when using it for the diagnosis of single-vessel, 54% for the diagnosis of two-vessel CAD, it demonstrates a higher sensitivity of 75% for triple-vessel disease, While left main stem disease of at least 50% stenosis has a very high negative predictive value of almost 99%, the positive predictive value is low with less than 15%. The sensitivity and positive likelihood ratio are 67% and 5.4, respectively. However, because of the small numbers, the results are not significant. It also demonstrates that when using 2D-Echo for the diagnosis of proximal LAD-CAD, the sensitivity is almost 62%, echocardiographic detection of double vessel disease with the involvement of the proximal LAD has a sensitivity and a positive predictive value of about 63% and 67 %, respectively.

DISCUSSION

This study included 300 people with slightly more men than women (164 and 136, respectively). The mean age of the study population was with 54.8 (±8.6)

Table 4. The Validity of 2D-Echocardiography by the Extent of Coronary Vessel Involvement.

Validity Measures	Single vessel disease	Double vessel disease	Triple-vessel disease	Left Main Stem Disease	PLAD Disease	2 Vessel Disease with one of them Being PLAD
Sensitivity	**49.21%**	**54.29%**	**75.00%**	**66.67%**	**61.54%**	**63.16%**
Specificity	**87.76%**	**87.76%**	**87.76%**	**87.76%**	**87.76%**	**87.76%**
Accuracy	**72.67%**	**78.95%**	**85.25%**	**87.13%**	**80.29%**	**80.88%**
PPV	**72.09%**	**61.29%**	**60.00%**	**14.29%**	**66.67%**	**66.67%**
NPV	**72.88%**	**84.31%**	**93.48%**	**98.85%**	**85.15%**	**86.00%**
Chi Square (χ^2) =	**26.76**	**25.5%**	**40.9%**		**34.99**	**36.47**
p-value	**<0.001**	**<0.001**	**<0.001**			
Fisher exact test p-value				**0.099**	**<0.001**	**<0.001**

years (18 to 72 years) slightly lower than the one in Elhendy's study who found a mean age of 57 ±13 years7 and the one performed in California (56.4 ±15.8 years).[9] A mean age of greater than 50 years in most studies is quite plausible since CAD is a disease that becomes more evident as people age, due to less people with CAD reach their 60s secondary to its fatal complications and lack of early diagnosis and the diverse socioeconomic status and their poor access to outpatient health care .[10] Our results confirm the trend noted in the Iraq Ministry of Health which states that there is a dramatic increase in heart diseases after the age of 50.[11] Regarding the gender distribution the ratio of men to women in this study was found to be 54.7% to 45.3%. Other studies had a similar ratio regarding gender like in Chang's study that found 59% of the study population to be men.[8] While (Table 1) reflects the distribution of CAD-severity among the study population according to the definition given in Patients and Methods, starting from (Table 2), a positive angiography was defined as significant or severe CAD while negative angiographic results were considered to be no CAD or non-significant disease. The results were grouped in order to simplify calculations and to give results and interpretations according to the generally accepted division of CAD being present or absent. Even though non-significant obstruction (1%-49% obstruction) was considered negative, a newer study shows that women with symptoms and signs suggestive of ischemia but with normal coronary arteries (stenosis of 0% in all coronary arteries) had a threefold risk for cardiovascular events, and those with nonobstructive CAD (stenosis in any coronary artery of 1%-49%) had a sixfold risk of cardiovascular events as compared to a control group of asymptomatic community-based women with no history of heart disease. The cardiovascular events were most frequent in women with four or more cardiac risk factors.[12] At least half of all first coronary events occur in asymptomatic individuals who are unaware that they have developed silent CAD, and substantial risk reduction can be attained with both secondary and primary prevention measures.[13] Scanlon and Faxon discovered that although coronary lesions that reduce luminal diameter by less than 50% are considered hemodynamically insignificant, they are not clinically benign.[14] Others suggests a direct correlation exists between the angiographic severity of coronary disease and the amount of angiographically insignificant plaque buildup elsewhere in the coronary tree and the higher mortality rate of patients with multi-vessel disease may occur because they have more mildly stenotic or non-stenotic plaques that are

potential sites for acute coronary events than those with one-vessel disease.[15]

The overall echo sensitivity and specificity were 58.4%, and 87.8%, respectively, which was a little lower than those found by a study performed by Chen who recorded an overall sensitivity of 2D-Echo of 67% with a specificity of 99%. They also suggest that the sensitivity is higher in patients with past MI than in those without MI (81% versus 42%), as would be expected.[16] The lack of standardization as to when a two-dimensional resting echocardiographic study is considered positive in the diagnosis of CAD contributes to the variation of sensitivity and spesificity in different studies, and due to the imaging windows which become more limited as body weight increases. Thus, it may be less accurate in the more obese populations.[17] The higher sensitivity and positive predictive value of 2D-Echo in men might be due to the gender differences in presentation and disease manifestations as atypical chest pain is more common in women.[4]

In this study, wall motion abnormalitiy was significantly associated with angiographic CAD ($\chi 2 = 69.3$, p-value < 0.001). Dortimer and Dejoseph discovered similar findings almost 35 years ago the explanation is that partially obstructed vessels cause diminished wall motion without actual MI.18 Kirk states that regional WMA suggests ischemia while the size of the defect and associated findings (e.g., left ventricular dilatation, global function, mitral regurgitation) reflect the level of clinical risk.[10] Elhendy suggests that resting wall motion abnormalities were the only predictor of an abnormal perfusion and its presence was an independent predictor of an ischemic response.[7]Others found that the resting wall motion score index appears to be a more powerful predictor of combined cardiovascular event than LVEF in patients evaluated for CAD and the resting WMA is indeed predictive of obstructive coronary disease,8 while the LVEF is well known prognostic marker for cardiovascular events. Even though WMA is significant for the diagnosis of CAD the examiner needs to be aware that artifacts can lead to a false interpretation.19 Although an expert can identify this artifact, at times it is difficult to separate it from an actual abnormality, especially when the rest of the posterior-inferior wall is not well seen. The ability to deal with such artifacts is a vital interpretative issue.[19] Furthermore the elderly diabetic patients unable to exercise with low EF and WMA appeared to be of highest risk of severe CAD.8In this study, coronary angiographic results show that more than 65% of the study population had CAD, about 45% significant and more than 20% severe disease. Our results indicate that patients without previous MI can have ischemic signs on echocardiography as Gibbons suggests that LV segmental wall motion abnormalities occur in patients with chronic stable angina and a history of previous MI.[4]

In this study, the multinominal logistic regression model of 2D-Echo findings according to likelihood ratio tests showed that echocardiographic findings such as WMA, left ventricular dysfunction, an ejection fraction < 50%, and LV dilatation correlate very well with angiographic results when present, being indicative of significant or severe CAD. However, their absence cannot exclude CAD. Other echo findings like mitral valve regurgitation ($p = 0.012$) and thinning of the septum ($p = 0.05$) show a less stringent association with angiographic results. When differentiating the angiographic results by the diseased vessels .The sensitivity as well as the negative predictive value rise from 50% and 73% (single-vessel disease) to 75% and 92% (triple-vessel disease), which means that according to this study's results, triple-vessel CAD can be excluded to a great extent in a patient with a negative 2D-Echo, yet, double- or single-vessel disease cannot be ruled out. Accordingly, significant three-vessel

disease can be detected more easily than significant single- or double-vessel disease by 2D-Echo.

When looking at LMS involvement with a stenosis of more than 50%, the validity measures are lower than the measures in triple vessel disease; however, the negative predictive value is higher with almost 99%. Yet, these values are limited by the fact that only 3 patients (1%) presented with LMS involvement, also represented by the non significant p-value of 0.099. Similarly, with proximal LAD narrowing the validity measures all are less as compared to those in three vessel disease .The reason for looking at LMS and proximal LAD involvement separately from two- and three-vessel disease is based on the knowledge that patients with severe stenosis of the left main coronary artery have a poor prognosis when treated medically and that the presence of severe proximal LAD disease significantly reduces the survival rate.[15]The five-year survival rate with three-vessel disease plus 95% proximal LAD stenosis is 59% compared with three-vessel disease without LAD stenosis being 79% .[4]

In the CASS registry of medically treated patients the 12-year survival rate of patients with normal coronary arteries was 91% compared with 74% for those with one-vessel disease, 59% for those with two-vessel disease, and 40% for those with three-vessel disease ($p < 0.001$). The effect of LV dysfunction on survival was quite dramatic. In the CASS registry, the 12-year survival rate of patients with ejection fractions in the range of 50% to 100%, 35% to 49%, and < 35% were 73%, 54%, and 21%, respectively ($p < 0.001$).[20]

As the severity progresses from single- to double-vessel-artery disease, the extent of coronary atheroma increases from 40% to 75% of the coronary tree; after this, the extent of coronary disease increases only slightly, even when multiple severe stenoses are present.[15]

CONCLUSIONS AND RECOMMENDATIONS

The overall two-dimensional echocardiographic findings can be helpful in ruling in significant or severe coronary artery disease. The negative predictive value is more useful in the women (31.08% vs. 65.63%). The best correlates with angiography were wall motion abnormality and left ventricular dysfunction .The more coronary arteries are diseased, the more two-dimensional echocardiography corresponds to coronary angiographic results. Further studies are needed to confirm the results of this study before two-dimensional resting echocardiography can be routinely used in the diagnosis of coronary artery disease.

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پوخته

هەلسەنگاندنا بکێرهاتنا ئیکو یا دلی بو دەستنیشانکرنا نەخوشیێن دەمارێن دلی

پێشەکی: نەخوشیێن دەمارێن دلی ئەگەرەکێ سەرەکی یێ مرنێ و توشبوونێ نە لسەرانسەرێ وەلاتێن جیهانێ و ل وەلاتێن پێشکەفتی و یێن ژنوی پێشدکەڤن سەرەرای پێشڤەچوونا زور دواریّ ئامرازێن دەستنیشانکرنێ و رێکێن چارەسەریێ دا. هەلسەنگاندنا کلینیکی یا نەخوشیّ دلی دەستپێدکەت ب وەرگرتنا پێزانینا وفەحسەکا تەمام یا کلینیکی, بەلێ گەلەك نەخوش پێدڤی ب تاقیکرنا نە بو دەستنیشانکرنا نەساخیێ وەکی نەخشا دلی یا سادە ە نەخشا دلی دگەل لڤینێ. کێم ڤەکولین هاتینە کرن لدور بەراوردکرنا نیشانێن ئێکو یا دلی دگەل قەستەرا دەمارێن دلی بو دەستنیشانکرنا نەخوشیێن دەمارێن دلی.

ئارمانج ژڤێ ڤەکولینێ: هەلسەنگاندنا بکێرهاتنا ئیکو یا دلی بو دەستنیشانکرنا نەخوشیێن دەمارێن دلی

رێکێن ڤەکولینێ: ئەڤ ڤەکولینا برگەیی هاتە ئەنجامدان لسەنتەرێ دلی یێ نەخوشخانا ئازادی یا فێرکرنێ لباژێرێ دهوکێ. پێزانین هاتنە هەرگرتن هەر ژ کانونا ئێکێ 2010 هەتا گولانا 2010ێ. ژمارا بەشدابوویا دڤێ ڤەکولینێ دا 300 نەخوش بوون کو ژیێ وان ژ 18 سالان پتر بوو و ئەو بوون یێن کو فەحسا ئیکو بو هاتییە کرن و هاتینە رەوانەکرن بو قەستەرا دەمارێن دلی (164 نێر بوون و 136 مێ).

ئەنجام: رێژا (86٪) ژ نەخوشان دژیێ 40–59 سالیێ دا بوون. سەرجەمێ هەستیاری و تایبەتمەندی و دروستیا فەحسا ئیکو بڤی رەنگی بوو: 58٪, 88٪, 68٪ لدویف ئێك. هندەك پێڤەرێن بکێرهاتنا ئیکو یێ دجیاواز بوون دناڤبەرا ئافرەت وزەلامان دا, بەلێ ئەڤ جیاوازییە نە د گرنگ بوون ژلایێ ئاماریّ ڤە. هندی پتر ەمارێن دلی دتوشبووی بان پتر نیشانێن ئیکویێ دگەل یێن قەستەری دچوون و ژ سەرجەمێ شەش نیشانێن ئیکویێ نەدروستیێن لڤینا دیواریّ دلی و لدویفدا نەدروستی دکاریّ رەخێ چەپێ یێ دلی ژهەمیان پتر پەیوەندی دگەل نەخوشیێن دەمارێن دلی هەبوو.

دەرئەنجام: تێکرایێ بکێرهاتنا فەحسا ئیکو بو دەستنیشانکرنا نەخوشیێن دەمارێن دل یا کێمە و تایبەتمەندییا وێ پترە ژ هەستیاریێ. پەیوەندی موکمترە دگەل ژمارا دەمارێن توشبووی و ژ نیشانا نەدروستیێن لڤینا دیواریّ دلی و نەدروستی دکاریّ رەخێ چەپێ یێ دلی پتر پەیوەندی هەبوو.

مصداقية فحص صدى القلب ثنائي الابعاد في تشخيص أمراض القلب التاجية

الخلفية : ان أمراض القلب التاجية هي احد الأسباب المهمة للمراضه والوفيات في كل من الدول المتطورة والنامية. ان التقييم السريري لالم الصدر يبدا بالتمحيص فى التاريخ المرضى اضافة الى الفحص السريري الدقيق الا ان العديد من الحالات تحتاج الى فحوص أخرى لغرض التثبت مثل مخطط كهربائية القلب وفحص تخطيط القلب بالإجهاد. مع ان الدراسات قد تحرت العلاقه بين امراض القلب التاجيه ونتائج فحص صدى القلب الا ان القليل منها قد اجرى مقارنه مباشره بين المعطيات المختلفه لفحص صدى القلب مع نتائج قسطرة الشرايين التاجيه كفحص مرجعي معتمد.

الهدف : تقدير مصداقية فحص صدى القلب ثنائي الابعاد في تشخيص أمراض القلب التاجية.

المنهجية : دراسة مقطعية أجريت في مستشفى أزادي العام التعليمي/ مركز القلب و قسطرة الشرايين التاجيه خلال الفترة من 20 / كانون الثاني إلى 8 / ايار من عام 2010. شملت الدراسة (300) مريض ادخلو على التعاقب منهم (164) ذكور و(136) إناث فوق 18سنة من الذين تم تحضيرهم لإجراء عملية قسطرة الشرايين التاجية وقد خضع جميعهم لفحص صدى القلب ثنائي الابعاد مسبقا.

النتائج : شكلت الفئة العمرية 40 – 59 سنة المجموعه الاكبر(86٪) فى عينة البحث. أظهرت نتائج الدراسة أن : (Sensitivity) و(Specificity)و(Accuracy)شكل عام هي كالأتي 58٪ , 88٪, 68٪ مع اختلافات فى النتائج بين الذكور والاناث لم تحقق فوا رق احصائيه باستثناء ال Negative predictive Value التي كانت اعلى لدى الاناث بفارق احصائي معنوي (p value < 0.01) . كما اظهرت النتائج بانه كلما ازداد عدد الشرايين المصابه كلما تحسنت ايجابية فحص صدى القلب. اما بالنسبة لمعطيات ا لفحص فقد تبين ان الحركة غير الطبيعيه لجدار القلب هى الاكثر ارتباطا بنتائج القسطره (p value < 0.0001) يليها الخلل الوظيفي للبطين الايسر(p value < 0.0001).

الاستنتاجات :ان الفحص ذو مصداقيه محدوده في المجال ا لتشخيصي ترتبط بعدد الشرايين المصابه وان اكثر معطيات الفحص ارتباطا بنتائج القسطره هي الحركة غير الطبيعيه لجدار القلب والخلل الوظيفي للبطين الايسر.

EFFECTS OF ANGIOTENSIN II ANTAGONIST LOSARTAN VERSUS ANGIOTENSIN CONVERTING ENZYME INHIBITOR ENALAPRIL ON SERUM URIC ACID LEVELS IN PATIENTS WITH METABOLIC SYNDROME

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ABSTRACT

Background and objectives: To investigate the effects of losartan and enalapril on serum uric acid in hypertensive patients with metabolic syndrome, one hundred and twenty six hypertensive patients, having markers of metabolic syndrome included in the study.
Methods : The patients were divided into two groups. Group 1 (60 patients) was given losartan (50 mg/ day) and group 2 (66 patients) was given enalapril (20 mg/ day). A control group of seventy apparently healthy individuals were included. Metabolic syndrome was diagnosed according to diagnostic criteria of metabolic syndrome related to the American National Cholesterol Education Program-Adult Treatment Panel III. Serum uric acid levels were measured before and after drug administration.
Results: The results revealed a significant higher levels of uric acid were found in the hypertensive patients as compared with control group and a significant drop of uric acid was noted after treatment with losartan but not with enalapril.
Conclusion: In conclusion, this study demonstrates significantly higher serum uric acid concentrations in hypertensive patients having markers of metabolic syndrome. Losartan but not enalapril therapy produced a significant fall in the serum uric acid level. Losartan can be useful therapeutic agent to control blood pressure and to reduce serum uric acid level in hypertensive patients having markers of metabolic syndrome and hyperuricaemia.

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Key words: metabolic syndrome, hypertension, uric acid, losartan, enalapril.

Some investigators have suggested that uric acid plays a causal role in the development of cardiovascular disease [1] whereas others have concluded that uric acid merely reflects other concomitant risk factors, such as hypertension, insulin resistance, obesity, or lipid abnormality [2]. Elevated serum uric acid concentrations are also found in healthy offspring of parents with coronary heart disease, indicating a possible causal relationship [3].

Krishnan et al [4] demonstrating that hyperuricemia increases the risk of developing hypertension by approximately 80%, independent of baseline blood pressure measurements, renal function, serum lipid levels, body mass index, proteinuria, alcohol use, and age. Johnson et al [5] reported that elevated uric acid level was observed in 40% to 60% of hypertensive subjects; similarly, hypertension was observed in 50% to 65% of subjects with gout. Johnson et al [6] reported that hyperuricemia was observed in 25% of treated hypertensive subjects, 50% of those without treatment, and 75% to 100% of those with malignant hypertension or renal dysfunction.

Serum uric acid (SUA) levels are often increased in subjects with MS. However, none of the proposed sets of diagnostic criteria include SUA levels in the definition of MS [7,8]. In 2001, the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) published the most widely used set of diagnostic criteria. These criteria include elevated plasma triglyceride (TRG) levels (≥150 mg/dl[1.69 mmol/l]), decreased levels of high-density lipoprotein

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cholesterol (HDL-C) (<40 mg/dl[1.04 mmol/l] in men and <50 mg/dl[1.29 mmol/l] in women), elevated blood pressure (BP)(≥130/85 mm Hg), increased fasting plasma glucose levels (≥ 110 mg/dl [6.1 mmol/l]), and abdominal obesity (waist circumference >102 cm in men and >88 cm in women) [9].

It is possible that the increased cardiovascular disease risk associated with the MS is partially attributed to elevated circulating SUA concentration [7,8]. Large epidemiologic studies demonstrated that the prevalence of MS showed a graded increase according to SUA levels [10,11,12]. Moreover, SUA concentration was positively correlated with blood pressure (BP), body mass index, levels of fasting plasma glucose, triglycerides, high-sensitivity C-reactive protein, and inversely correlated with high density lipoprotein cholesterol levels (HDL-C) [8].

Many drugs have hypouricaemic properties, in addition to their main therapeutic effects. The oral weight loss agent sibutramine decreases serum uric acid in obese patients by 20% to 25% [13]. Similarly, in patients with type 2 diabetes and hyperuricemia, the insulin sensitizing agent troglitazone lowers serum uric acid by 20% to 25% [14]. Ramipril was found to increase the excretion of uric acid in a number of hypertensive patients [15].

The present study was conducted to investigate the effects of losartan compared with enalapril on uric acid levels in hypertensive patient having markers of metabolic syndrome.

METHODS

One hundred and twenty six hypertensive patients having markers of metabolic syndrome participated in this study. They were divided into two groups according to the type of the drug taken. Group 1 was given losartan (Angizar 50mg, Micro Pharmaceutical Industries, Co. Ltd., India) in doses of 50mg daily. They are 28 males and 32 females, with a mean age of 56.68±6.32 years. Group 2 received enalapril (Enalapril 20mg, Asia Pharmaceutical Industries, Co. Ltd., Aleppo-Syria) in doses of 20 mg once daily. They are 30 males and 36 females with mean ages of 52.80±7.23 years. Another 70 healthy, non obese, normotensive individuals, age and gender matching with study patients, participated in the study as a control group. They were 34 males and 36 females, with mean age of 53.51±6.66 years.

This open 2-month, controlled, comparative clinical trial was conducted on hypertensive patients having markers of metabolic syndrome who were seen at Ibn-Sina teaching hospital in Mosul, Iraq. The study protocol was approved by the Ethics Committees of the College of medicine and Mosul health administration.

Non-diabetic patients with mild hypertension (Stage 1: Systolic 140 - 159 mmHg and Diastolic 90 - 99 mmHg) [16], who met the diagnostic criteria of metabolic syndrome according to the American National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III) [9] were included in this study. Those with hepatic or renal diseases, pregnancy and lactation, hypertensive patients on antihypertensive therapy, hypersensitive patients on losartan or enalapril, gouty patients and inflammatory diseases such as rheumatoid arthritis were excluded.

Markers of metabolic syndrome including, waist circumference, blood pressure, serum glucose concentration, triglycerides, and HDL-cholesterol were determined before and at the end of study period. The presence of 3 or more of such markers indicates metabolic syndrome state. Blood pressure was measured by standard mercury sphygmomanometer. Goal BP after treatment was less than 140/ 90 mmHg. Serum glucose concentration, total cholesterol, triglycerides, and HDL-cholesterol were measured by using special kits. LDL-cholesterol was calculated from Friedewald equation [17].

Serum uric acid concentration was measured at baseline and after 2 months therapy with losartan or enalapril by enzymatic method using a kit supplied by Biolabo laboratories (France).

Statistical methods: Standard statistical methods were used to determine the mean and standard deviation (SD). Paired student t-test was used to compare the results between before and after drug therapy. Unpaired t-test was used to compare the results of cases before and after drug therapy with control. The statistical results were considered significant at p=0.05 or less.

RESULTS

Baseline measurement of waist circumference, Body mass index (BMI) , blood pressure, serum glucose concentrations and lipid profile of the patient's groups showed a significant elevation as compared with the control group, while HDL-cholesterol showed lowered values as compared with the controls (P<0.001) (Table 1).

Baseline uric acid levels were 306.69±67.72 µmol/l for losartan group and 302.94±56.86 µmol/l for enalapril group which showed a significant elevation (P<0.001) as compared with the control (284.95±76.52 µmol/l) (Table 1 and Table 3) respectively. Comparison of uric acid levels before and after 2 months of therapy by each drug showed a significant reduction in losartan group (P<0.001) (Table 2) but not in enalapril group (p=0.123) (Table 4). Comparison of uric acid levels between losartan group and enalapril group showed a significant reduction in the losartan group (P<0.001) as compared with the enalapril group (Table 5).

Table 1. Comparison of data between control and losartan group (before and after therapy).

Parameters	Mean ± SD		
	Control (n=70)	Before (n=60)	After (n=60)
BMI (kg/m2)	22.2 ± 1.8	33.46 ± 2.08***	30.95 ± 1.8***
Waist circum. (cm)	83.95 ± 6.2	106.79 ± 8.53***	104.08 ± 8.3***
SBP (mm Hg)	127.05 ± 6.93	143.60 ± 7.72***	136.82 ± 8.4***
DBP (mm Hg)	79.24 ± 4.91	92.18 ± 6.21***	83.92 ± 6.3***
FSG (mmol/L)	4.75 ± 0.9	6.6 ± 0.4***	5.12 ± 0.7***
Total-C (mmol/L)	4.45 ± 0.63	5.28 ± 0.74***	4.65 ± 0.8***
TG (mmol/L)	1.48 ± 0.6	1.67 ± 0.37	1.23 ± 0.5*
HDL-C (mmol/L)	1.60 ± 0.28	1.32 ± 0.32***	1.54 ± 0.4***
LDL-C (mmol/L)	2.20 ± 0.70	3.20 ± 0.67***	2.84 ± 0.8***
Uric acid (□mol/L)	284.95 ± 76.52	306.69 ± 67.72	275.92 ± 61.63

** Significant difference from control at p<0.05, ** at p<0.01 and *** at p<0.001 using unpaired t-test.*

Table 2. Comparison of the effects of losartan before and after therapy .

Parameters	Mean ± SD		
	Before (n=60)	After (n=60)	p-value
BMI (kg/m2)	33.46 ± 2.08	30.95 ± 1.8***	<0.001
Waist circum. (cm)	106.79 ± 8.53	104.08 ± 8.3***	<0.001
SBP (mm Hg)	143.60 ± 7.72	136.82 ± 8.4***	<0.001
DBP (mm Hg)	92.18 ± 6.21	83.92 ± 6.3***	<0.001
FSG (mmol/L)	6.6 ± 0.4	5.12 ± 0.7***	<0.001
Total-C (mmol/L)	5.28 ± 0.74	4.65 ± 0.8	0.135(NS)
TG (mmol/L)	1.67 ± 0.37	1.23 ± 0.5	0.240(NS)
HDL-C (mmol/L)	1.32 ± 0.32	1.54 ± 0.4***	<0.001
LDL-C (mmol/L)	3.20 ± 0.67	2.84 ± 0.8	0.098(NS)
Uric acid (□mol/L)	306.69 ± 67.72	275.92 ± 61.63***	<0.001

****Significant difference at p<0.001 using paired t-test. NS= Not significant.*

Table 3. Comparison of data between control and enalapril group (before and after therapy).

Parameters	Mean ± SD		
	Control (n=70)	Before (n=66)	After (n=66)
BMI (kg/m2)	22.2 ± 1.8	32.79 ± 1.9***	30.6 ± 2.18***
Waist circum. (cm)	83.95 ± 6.2	103.44 ± 8.87***	100.8 ± 8.53***
SBP (mm Hg)	127.05 ± 6.93	145.78 ± 5.39***	136.95 ± 7.58***
DBP (mm Hg)	79.24 ± 4.91	91.44 ± 6.15***	86.07 ± 5.0***
FSG (mmol/L)	4.75 ± 0.9	6.55 ± 0.38***	5.35 ± 0.66***
Total-C (mmol/L)	4.45 ± 0.63	5. 40 ± 0.93***	5.42 ± 0.76***
TG (mmol/L)	1.48 ± 0.6	1.36 ± 0.60	1.2 ± 0.51*
HDL-C (mmol/L)	1.60 ± 0.28	1.40 ± 0.3***	1.57 ± 0.32***
LDL-C (mmol/L)	2.20 ± 0.70	3.26 ± 0.72***	3.27 ± 0.99***
Uric acid (□mol/L)	284.95 ± 76.52	302.94 ± 56.86	289.99 ± 50.28

** Significant difference from control at p<0.05, ** at p<0.01 and *** at p<0.001 using unpaired t-test*

Table 4. Comparison of the effects of enalapril before and after therapy .

Parameters	Mean ± SD		
	Before (n=60)	After (n=60)	p-value
BMI (kg/m2)	32.79 ± 1.9	30.6 ± 2.18***	<0.001
Waist circum. (cm)	103.44 ± 8.87	100.8 ± 8.53***	<0.001
SBP (mm Hg)	145.78 ± 5.39	136.95 ± 7.58***	<0.001
DBP (mm Hg)	91.44 ± 6.15	86.07 ± 5.0***	<0.001
FSG (mmol/L)	6.55 ± 0.38	5.35 ± 0.66***	<0.001
Total-C (mmol/L)	5. 40 ± 0.93	5.42 ± 0.76	0.205(NS)
TG (mmol/L)	1.36 ± 0.60	1.2 ± 0.51	0.193(NS)
HDL-C (mmol/L)	1.40 ± 0.3	1.57 ± 0.32	0.178(NS)
LDL-C (mmol/L)	3.26 ± 0.72	3.27 ± 0.99	0.716(NS)
Uric acid (□mol/L)	302.94 ± 56.86	289.99 ± 50.28	0.132(NS)

****Significant difference at p<0.001 using paired t-test. NS= Not significant.*

Table 5. Comparison of data after losartan and enalapril therapy.

Parameters	Losartan (n=60)	Mean ± SD Enalapril (n=66)	p-value
BMI (kg/m2)	30.95 ± 1.8	30.6 ± 2.18	0.026 (NS)
Waist circum. (cm)	104.08 ± 8.3	100.8 ± 8.53	0.605(NS)
SBP (mm Hg)	136.82 ± 8.4	136.95 ± 7.58	0.134 (NS)
DBP (mm Hg)	83.92 ± 6.3	86.07 ± 5.0*	0.05
FSG (mmol/L)	5.12 ± 0.7	5.35 ± 0.66*	0.05
Total-C (mmol/L)	4.65 ± 0.8	5.42 ± 0.76	0.120(NS)
TG (mmol/L)	1.23 ± 0.5	1.2 ± 0.51	0.321(NS)
HDL-C (mmol/L)	1.54 ± 0.4	1.57 ± 0.32	0.062(NS)
LDL-C (mmol/L)	2.84 ± 0.8	3.27 ± 0.99	0.126(NS)
Uric acid (□mol/L)	275.92 ± 61.63	289.99 ± 50.28***	<0.001

** Significant difference at p<0.05 and *** at p<0.001. NS= Not significant.*

DISCUSSION

The present study demonstrates significantly higher uric acid levels in subjects with metabolic syndrome as compared with the control group. These results are in consistent with the results obtained from many articles which also demonstrate increased levels of uric acid in patients with metabolic syndrome [8,12,18]. The increase in serum uric acid in metabolic syndrome may be related to insulin resistance, which is accompanied MS.

Several mechanisms were attributed to the increase of UA levels in MS. One of these

mechanisms which is reported by Cappuccio et al [19] , is related to insulin resistance, which is accompanied by MS. Proximal tubular reabsorption of UA occurs by an active transport mechanism closely linked to or identical with the tubular reabsorption of sodium. Insulin can enhance renal tubular sodium reabsorption in humans. Furthermore, renal excretion of UA is reduced in situations with increased renal tubular reabsorption of sodium. Another mechanism for the increased SUA levels in MS is that MS is associated with increased oxidative stress [20] . Because uric acid is considered to be an effective antioxidant. The elevated SUA levels to unique biochemical properties of losartan [22,23,24]. The hypouricemic effect of losartan may be due to that losartan target the urate anion exchange and diminish urate reabsorption in the proximal convoluted tubule; as a result, the urate excretion fraction is increased by 13%-30% and increases renal uric acid excretion [25].

This aspect of losartan therapy might have therapeutic advantages by reducing the risk of elevated uric acid in patient with MS, since elevated serum uric acid levels in patient with MS is regarded as a risk factor for the development of CV diseases [26] and may ameliorate hyperuricemia induced by other drugs. It was reported that the risk of death due to ischemic heart disease increased by 77% (men), and by 30% (women) when serum uric acid levels where in the highest quartile (>416 µmol/l) compared with the lowest quartile (<321 µmol/l) [27].

Data obtained from the present study showed that enalapril produce no significant effects on uric acid concentration in patients with metabolic syndrome. Data from the literature demonstrates different results. No effect was reported by Tikkanen et al., [28] , rise in SUA levels reported by De Rosa et al [29] , and and others demonstrates SUA lowering effect [8,30] .

encountered in individuals with MS may reflect a compensatory mechanism counteracting the increased oxidative stress associated with the MS [21].

In the present study, only losartan causes a significant reduction of serum uric acid concentrations in patients with metabolic syndrome after 2 months of therapy. These results indicate that losartan have uricosuric effects. Many studies have demonstrated that the uricosuric effect of losartan was due to the parent compound and not to the active metabolite EXP 3174 and that this effect is independent of angiotensin II receptor blockade and is due

CONCLUSION

This study demonstrates significantly higher serum uric acid levels in hypertensive patients having markers of metabolic syndrome. losartan therapy but not enalapril therapy produced a significant fall in serum uric acid levels. Losartan can be a useful therapeutic agent to reduce serum uric acid level in hypertensive patients having markers of metabolic syndrome and hyperuricaemia.

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پوختە

کارتێکرنا ئەنتاگونستێ ئەنجیوتنسین 2 (Isosartan) بەرامبەر (Enalapril) ل سەر ریژا (Urice Acid) د خوینێدا د نەخوشێن توشی سندروما میتابولیکی بوین

پێشەکی و ئامانجەکان: ژ بو بەراوردکرنا هەردو دەرمانێن (لوسارتان) و (ئینالابریل) ل سەر ریژا (Urice Acid) د خوینا (126) نەخوشێنێ ژ جورێ سڤك توشبوییێ ب زێدەبونا فشارا خوینێ و سندروما میتابولیکی بوین.

ڕێکێن ڤەکولینێ: کوژمێ نەخوشان هاتە دابەشکرن بو دوو گروپان ل دویف جورێ چارەسەریێ. گروپێ ئێکێ (60) نەخوش (40) ملیگرامێن روچانە ژ دەرمانێ لوسارتان بو هاتە دان بو ماوێ دوو هەیڤان و گروپێ دووێ (66) نەخوش (20) ملیگرامێن روژانە ژ دەرمانێ ئینالابرین هاتە دان بو ماوێ دوو هەیڤان. (70) خوبەخشێن ساخ و سەلیم وەك گروپێ کونترول هاتە ژێگرتن. پیڤەرێن پروگرامێ ئەمریکی یێ نەتەوی بو ریژێن کولیترولێ هاتە ب کارئینان ب مەبەستا دەستنیشانکرنا سندرومێ میتابولیکی هەروەسا ریژا (Urice Acid) هاتە کێشان بوو هەردوو گروپێن نەخوشان بەری و پشتی دانا دەرمانی دگەل گروپێ کونترول.

ئەنجام: ئەنجامێن ڤەکولینێ جیاوازیەکا بەرچاڤ دیار کر د ریژا (Urice Acid) ل دەف نەخوشێن توشی فشارا خوینێ بوین بەرامبەر گروپا کونترول و هەر وەسا دابەزینەکا بەرچاڤ د ریژا (Urice Acid) ی دا پشتی چارەسەرکرنێ ب دەرمانێ لوسارتان بەرامبەر ب کارئینانا دەرمانێ ئەنالابریل

دەرئەنجام: ئەڤێ ڤەکولینێ دیار کر کو ریژەکا بلند و یا بەرچاڤ یا (Urice Acid) ی یا هەی ل دەف نەخوشێن توشی فشارا خوینێ یا بلند بوین و ب تایبەت یێن نیشانا سندروما میتابولیکی هەین. چارەسەرکرن ب دەرمانێ لوسارتان (نەك دەرمانێ ئینالابریل) بو ئەگەرێ دابەزینەکا بەرچاڤ د ریژا (Urice Acid) دا د خوینا نەخوشان دا، ژ بەر ڤێ چەندێ دەرمانێ لوسارتان یێ ب مفایە بو کونترولکرنا فشارا خوینێ و دابەزینا ریژا (Urice Acid)ی ل دەف نەخوشێن توشی فشارا خوینێ و نیشانێ سندرومێ میتابولیکی بوین و ب تایبەت ئەوێن ریژا (Urice Acid) لدەف وان زور بلند بوین.

الخلاصة

تاثيرات مثبط الأنجيوتنسين II اللوسارتان مقابل مثبط الإنزيم المحول للأنجيوتنسين الانالابريل على مستويات الحامض البولي في مصل الدم لدى المرضى الذين يعانون من متلازمة التمثيل الغذائي

خلفية البحث والاهداف: لتحري تأثيراتِ عقاري اللوسارتان والإنالابريل على مستوى الحامضِ البولي في مصل الدم لدى مرضى ارتفاع ضغط الدمِّ والمتلازمة الأيضيةِ، أجريت هذه الدراسة على 126 مريضا شخصوا حديثا إصابتهم بالضغط العالي من النوع الخفيف ولديهم علامات المتلازمة الأيضيةِ.

طرق البحث: قسمت مجموعة المرضى إلى مجموعتان حسب العلاج المعطى لهم. أعطيت المجموعة الأولى عقار اللوسارتان 50 ملغ يوميا، والمجموعة الثانية عقار الإنالابريل 20 ملغ يوميا، .أستغرقت فترة العلاج مدة شهرين. تم إختيار 70 شخصا سليما من المتطوعين (يبدون أصحاء) طبيعي الضغط ليكونوا مجموعة الضبط. شخصت المتلازمة الأيضية حسب معايير البرنامج الوطني لتعليم الكولسترول الأمريكي. تم قياس مستوى الحامض البولي لكل من مجموعة المرضى(قبل وبعد العلاج) ومجموعة الضبط.

النتائج: أظهرت النَتائِج ارتفاعا معنويا ملحوظا في مستوى الحامضِ البوليِ في مصل الدم لدى مرضى ارتفاع ضغط الدمِّ بالمقارنة مع مجموعة الضبط وانخفاضا معنويا في مستوى الحامضِ البوليِ بعد المعالجة بعقار اللوسارتان لكن لَيسَ مَع عقار الأنالابريل.

الاستنتاجات: في الاستنتاجات, أظهرت هذه الدراسةِ أن هناك ارتفاعا معنويا ملحوظا في مستوى الحامضِ البوليِ في مصل الدم لدى مرضى إرتفاع ضغط الدمِّ والذين لديهم علاماتُ المتلازمةِ الأيضيةِ. أدى العلاج بعقار اللوسارتان لكن لَيسَ العلاجَ بعقار الأنالابريل انخفاضا معنويا ملحوظا في مستوى الحامضِ البوليِ في مصل الدم. يُمكنُ أنْ يَكُونَ اللوسارتان علاجّاً مفيداً للسَيْطَرَة على ضغطِ الدمّ ولتَخفيض مستوى الحامضِ البولي في مصل الدم في مرضى إرتفاع ضغط الدمِّ والذين لديهم علاماتُ المتلازمةِ الأيضيةِ وفرط الحامض البولي في الدم.

A NOVEL NANO-CALCIUM CARBONATE-POLYURETHANE-BASED ROOT CANAL OBTURATION MATERIAL: SYNTHESIS AND EVALUATION OF SOME PHYSICAL PROPERTIES

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ABSTRACT

Objectives: The purpose of this study was to prepare a new root canal obturating material named Nano-Calcium Carbonate-Polyurethane and evaluate three of its physical properties which are solubility, water sorption and radiopacity.
Methods: Poly Carbonate, 1,6-Hexanmethylene Diisocyanate, NCO 49.79%, and 1,4-butanediol were mixed to form Polycarbonate based thermoplastic polyurethane. Additives material like Nano calcium carbonate powder, zinc oxide, calcium hydroxide and barium sulfate with different ratios blend together with the polycarbonate based thermoplastic polyurethane to form the final obturating material. Solubility water sorption and radiopacity tests have been done to evaluate some physical properties of this material.
Result and conclusion: The Nano-Calcium Carbonate-Polyurethane is a promising root canal filling material and their solubility percent, water sorption and radiopacity are consistent with ISO standards.

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Key words: Polyurethane, Nano-Calcium carbonate, Root canal obturation, Water sorption, solubility, Radiopacity

The success of endodontic therapy depends not only on adequate access and thorough biomechanical preparation but also on proper obturation. Several techniques and materials have been used since time immemorial for root canal obturation. The most popular and tested materials of choice are gutta percha and resilon. However gutta percha had its own advantages and disadvantages. Some of the disadvantages are lack of bonding to root dentin leading to micro leakage, increased shrinkage when used in the form of thermo plasticized material and non-re-inforcement of the root structure. Resilon has been introduced as a superior alternative to gutta percha. This synthetic polymer reportedly, not only provides a better seal but also reinforces the tooth structure through a combination of primer, dual cure sealer and resin obturating material [1]. The polyester chemistry containing bioactive and radio opaque fillers have been developed and tested. It performs, handles and looks like gutta-percha; in addition, when used in conjunction with a resin based sealant or bonding agent it forms a monoblock within the canals that bonds to the dentinal walls. However, there are some disadvantages for resilon such as low push-out bond strength [1] and low cohesive strength plus stiffness [2] .In addition, Resilon could not achieve a complete hermetic apical seal [3] .These results indicate that a more appropriate material for root canal obturation still needs to be developed. Lee et al, [4] developed a new polyurethane-based composite to serve as a root canal obturation material and a visible-light curable urethane-acrylate/tripropylene glycol diacrylate (UA/TPGDA) oligomer to serve as a root canal sealer. This material has excellent properties but the major disadvantage was that it composed

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chemically of Polybutyleneadipate polyol(PBA) which decomposed by time [4]. So, for long run this material may lose some of its physical and mechanical properties.

poly carbonate is a stable polyol and last for long time without biological disintegration .Calcium carbonate nano powder gave good properties when mixed with polyurethane. The major intention of this study is to prepare root canal filling material by using poly carbonate polyol in combination with calcium carbonate nano powder and other additive to enhance the physical properties of the previously prepared thermoplastic polyurethane obturating material [5]. The absorption of water by polymers is a phenomenon of considerable importance since it is accompanied by dimensional changes, also it reduces the tensile strength of the material. Regarding the solubility, it represents the mass of soluble materials from the polymers and the soluble material may affect the periapical tissue [6]. More, over the root canal filling material should present enough radiopacity to allow distinction from the adjacent anatomical structures , such as bone and tooth. Finally, The aims of this study are to prepare a new Nano-Calcium Carbonate-Polyurethane-based Root Canal–obturation Material and to evaluate some of the physical properties which are solubility, water sorption and radiopacity.

METHODS

1. Preparation of Nano CaCO3/TPU Composite as a Root Canal–Filling Material

PolyCarbonate(POLY-®CD220,Carbonic acid, dimethylester, polmer with1,6-hexandiol. MW 2000. Arch Chemicals,Inc,USA),1,6-Hexanmethylene Diisocyanate, NCO 49.79%, (HDI,Bayer MaterialScience,USA), and 1,4-butanediol(1,4-BD, Alfa Aesar,USA) were mixed in 1:1.12:0.1 molar ratios, dissolved in acetone (Acetonen99.5%, Sigma-Aldrich, USA) and reacted to form Polycarbonate based TPU. All chemicals used in this study are listed in (Table 1) and (Table 2).

Table 1. Raw materials

Designation	Composition	Supplier
Polyols		
POLY-CD®CD220 (PCA)	**Carbonic acid, dimethyl ester, polymer with 1,6-hexandiol.MW 2000, OH-number 55.6**	**Arch Chemicals, Inc.USA**
Chain extender		
1,4 BD	**1,4 –Butane diol equivalent weight 45 (MW 90)**	**Alfa Aesar,USA**
Isocyanate		
DESMODUR H (HDI)	**Hexamethylene-1,6-Diisocyanate, NCO %49.79.**	**Bayer MaterialScience,USA**
Catalyst		
DABCO®T-12 (0.1%)	**Dibutyltin dilaurate**	**Air Products,USA**
Solvent		
Acetone	**Aceton C3H6O 99.5%**	**Sigma –Aldrich,USA**

Table 2. Additives Raw Materials

Designation	Composition	Supplier
Zinc Oxid (ZnO)	ACS reagent ≥99.0%	Sigma- Aldrich
Barium Sulfate (BaSo4)	Reagent plus 99%	Sigma-Aldrich
Calcium hydroxide Ca(OH)2	ACS reagent ≥95.0%	Sigma –Aldrich
Calcium Carbonte Nano particles (CaCo3)	15-40 nm surface modified for adhesives	Sky Spring Nanomaterials, Inc.

Polycarbonate Polyol and chain extender were checked for H 2O content using Karl –Fisher device and water content was in the range of 0.01-0.05. Isocyanate was used as received from the supplier and isocyanate content was determined by the Di-n-butylamine method. The NCO content was 49%. All other additives ingredient were used as received. The polymerization reaction was carried out in 600 ml reaction cattle which was equipped with a mechanical stirrer, thermocouple, heater, Nitrogen inlet and Refux condenser.

Polycarbonate polyol was weighted and added to reaction cattle, then 1,4 BD was added , followed by the catalyst . Then acetone was added to the mixture and mixed with a stirrer. The reaction mixture was heated up to 50◦C and the HDI was added via funnel. After addition of HDI, the funnel was rinsed with a small amount of acetone and the reaction was continued for 2 hours at 50◦C.

During the synthesis, additional amount of acetone was added due to the high viscosity of polymer solution. After 2 hours of synthesis, clear viscous polymer solution was obtained. After polymerization is completed, a sample for NCO% determination was taken and NCO% was determined.

2-Additives mixing ratio: Filler materials shown in (Table 3) were added to the solution of polyurethane in the following ratios: 50 wt% of polyurethane solution and 50% fillers to form CaCo3/TPU composite .

Water sorption and Solubility

Specimen preparation:

A total of 10 discs for the new materials were made. Each specimen disc was 20 mm in diameter and 1.5 ± 0.1 mm thick which prepared using a metal mould. The material was prepared, by filling the mold with the material using a plastic spatula to condense, and covering it with a piece of polyester transparent film which was placed below and over the mold.

Table 3 . Weight percentage of the fillers

%wt of additives	Calcium Carbonate	Zinc Oxide	Calcium Hydroxide	Barium sulfate
50%	12	25	10	3

Test procedure

The specimens were transferred to the desiccators containing silica gel, freshly dried for 5 hours (h) at 130 ◦C. They were maintained in the desiccators at 37 ± 1 ◦C. After 24h, the specimens were removed and stored in a second desiccators which contained silica gel (freshly dried for 5h at 130 ◦C) and stored at the lower temperature (room) of 23 ± 1 ◦C for 1h. The specimens were weighed using an analytical balance (Mettler Analytical Balance, Gallenkamp Mettler, E. Mettler, Zurich, Switzerland) to an accuracy of ±

0.1 mg. This cycle was repeated until a constant mass (m1) was obtained, i.e. until the mass loss of each specimen was not more than 0.2 mg in any 24h period. The specimens were immersed in distilled water , and maintained at 37 ◦C for seven days. After that time, the specimens were removed, washed with water, surface water blotted away until free from visible moisture, and waved in the air for 15 seconds, then finally weighed 1 minute after being removed from the water. This mass (m2) was recorded. The specimens were placed in the desiccators using the same cycle as described above to obtain (m1). This cycle was repeated until constant mass (m3) was obtained. Finally, the specimens were measured for thickness. This was done by taking three readings in the centre of the specimen to measure the thickness. The mean values of thickness of each specimen, was used to calculate the volume (V) in cubic millimeters [6] .

Calculations and expression of results

The values of water sorption WSP , were calculated in micrograms per cubic millimeter for each of the specimens, by using the following equation:

WSP = (m2 – m3)/ V Where: m2 is the mass of the conditioned specimen in micrograms, after immersion in water for seven days; m3 is the reconditioned mass of the specimen in micrograms, and V is the volume of the specimen in cubic millimeters.

The values of water solubility WSL were calculated in micrograms per cubic millimeter for each specimen using the following equation:

WSL = (m1 – m3) / V where: m1 is the conditioned mass in micrograms;

m3 is the reconditioned mass of the specimen in micrograms, and V is the volume of the specimen n cubic millimeters [(6)] .

Radiopacity

A washer of 10mm internal diameter and 1mm height is filled with the mixed material and radiographed together with an aluminum step wedge having incremental thickness of 1 mm to 9 mm. The radiopacity of ten specimens is compared with that of the step wedge by means of a densitometer (Heiland electronic, Wetzler, Germany,(Figure 1).

The minimum requirement is 6 mm Al-equivalents, which may be on the low side considering that conventional gutta-percha points are about 6mm Al-equivalents. Most materials are in the range of 4–9 mm (ANSI/A.D.A Specification No.78) [(7)] .

Figure 1. Densitometer, Some specimens, and Aluminum step wedge that were used for Radiopacity test.

RESULTS

Solubility and water sorption:

The solubility of Nano calcium carbonate polyurethane material in micrograms per cubic millimeter was 0.0035 ±.0003, while the water sorption was 0.0047±.001. The allowable ratio for solubility for our material according to ISO specification is 0.026 g which represents 3% of the total weight which is 0.869 g. As shown in (Table 4) and (Figure 2).

Radiopacity

(Tables 4& 5) shows the mean gray value and equivalent aluminum thickness (mm) of Nano calcium carbonate polyurethane. Radiopacity was expressed in milimeters of aluminum and higher value represented greater radiopacity. Nano calcium carbonate polyurethane possessed radiopacity equal to 0.92 mm which is closest to the 0.93, the score of aluminum with 6 mm, complying with the ISO requirements. (Figure 3)

Table 4. The result of solubility percent and water sorption of Nano calcium carbonate polyurethane material in micrograms per cubic millimeter

	N	Minimum	Maximum	Mean	Std. Deviation
weight one (M1)	10	.576	1.079	.869	±.147
weight two (M2)	10	.577	1.088	.875	±.149
weight three (M3)	10	.563	1.059	.851	±.145
Volume of cylinder =r2*height *3.14	10	3.45	6.500	5.105	±.880
Water sorption	10	.004	.007	.0047	±.001
Water solubility	10	.003	.004	.0035	±.000

Table 5. The mean and standard deviation of radiopacity of Nano calcium carbonate polyurethane.

Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Mean	Standard deviation
0.92	0.89	0.84	1.07	0.89	0.89	1.07	0.84	0.89	0.92	0.92	±0.02

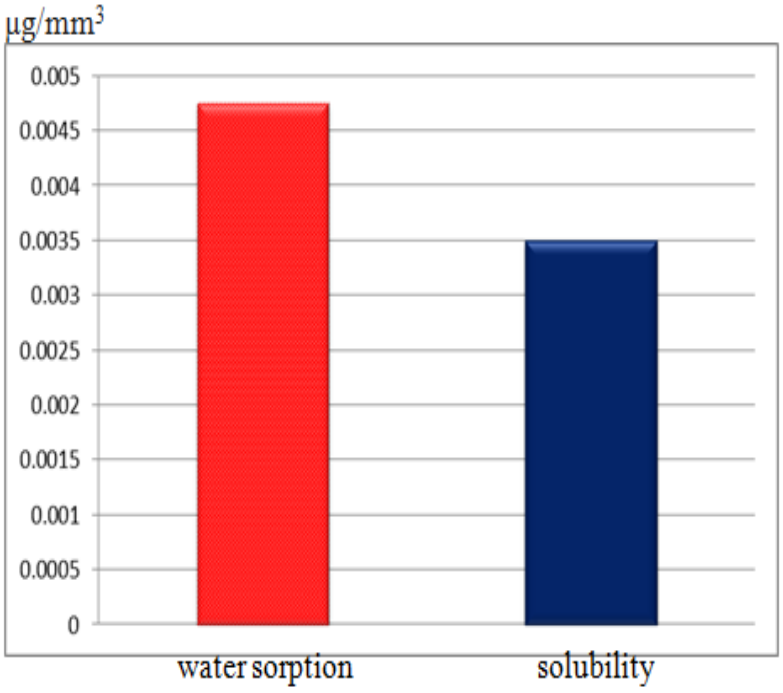
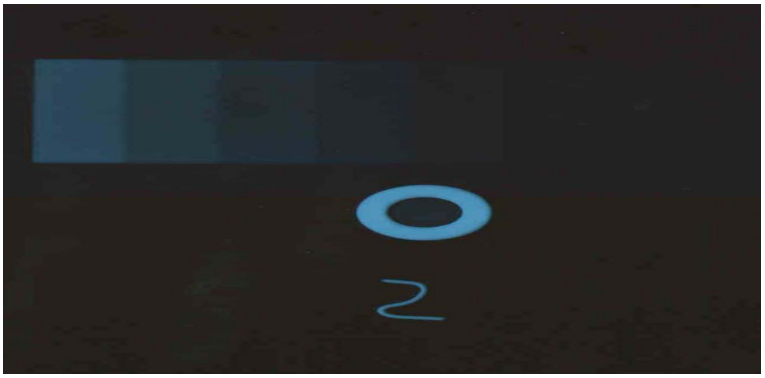


Figure 2: The result of solubility and water sorption of Nano calcium carbonate polyurethane material in micrograms per cubic millimeter.

A



B

Figure 3. A and B: The mean gray value and equivalent aluminum thickness (mm) of Nano calcium carbonate polyurethane

DISCUSSION

There is no ideal root canal filling material which fulfills all the requirements of successful endodontic treatment until now. Preparation of new root canal material that overcome the drawbacks of previous material is a logical demand [7]. The polyol which is used for the thermoplastized polyurethane prepared by Lee et al. [4] had short life span and it was liable for biological disintegration. The major intention of this study is to prepare root canal filling material by using poly carbonate polyol, which has very good stability for long duration, in combination with calcium carbonate nano powder and other additives to enhance the mechanical and physical properties of previous material [9].

ISO and ANSI/ADA have standardized some technological tests to investigate the physical properties of endodontic filling materials. Assessment of the radiopacity, solubility and water sorption properties in this study were evaluated as recommended by ISO standard (4049:1988). It appears from the results of water sorption and solubility that Nano calcium polyurethane material behaved satisfactory with this standard. Solubility is an undesirable property for a root canal filling because it can cause the filling to release components that may be biologically incompatible and formation of gaps can affect the hermetic seal of the root canal filling negatively. According to ISO standards the solubility of root canal filling shouldn't exceed 3% mass fraction. The value of solubility of Nano calcium carbonate polyurethane was within this limit which is 0.4% [6].

It's obvious that polymers are prone to hydrolysis by enzymes, mechanical loading, and water. The degradation products and their effects on oral tissues are of prime concern.

Resilon showed exposure of glass-filler particles following surface dissolution of the polymer matrix by a gravimetric analysis and SEM, creating a rough surface topography after incubation in lipase PS (from Burkholderia cepacia; Amano Enzyme Inc., Nagoya, Japan) or cholesterol esterase (from Pseudomonas species; Amano Enzyme Inc.) for 96 hours. Similarly, the presence of spherical polymer droplets that appeared deformed, pitted or much reduced in dimensions was seen with Resilon after enzymatic hydrolysis. Rates of hydrolysis of Resilon by lipase PS and cholesterol esterase were much faster than those of polycaprolactone at a 1×or even 4× enzyme concentration. Field-emission SEM and energy dispersive spectrometric analyses showed that the surface resinous component of Resilon was hydrolyzed after 20 minutes of sodium ethoxide immersion, exposing the spherulitic polymer structure and subsurface glass and bismuth oxychloride fillers. More-severe erosion occurred after 60 minutes of sodium ethoxide treatment, while gutta-percha was unaffected [9].

Furthermore, gutta-percha exhibited minimal surface changes after 4 months of incubation in wet dental sludge, while polycaprolactone and Resilon exhibited severe surface pitting and erosion. In the latter, disappearance of the polymer matrix was accompanied by exposure of mineral and bioactive glass fillers. Bacteria and hyphae-like structures were present on the Resilon surfaces [9].

Radiopacity is widely acknowledged as a desirable e property of all intraoral materials, including the endodontic root canal material. The root canal filling material must be radiopaque in order to detect the extention and the quality of the filling. Beyer-Olsen & Orstavik established a standardized system to measure the quality of radiopacity. They used an aluminum step-wedge with 2 mm increments as a reference to determine the equivalent aluminum thickness of the studied materials. In literature usually, conventional radiographic films and optical densitometers were used to evaluate the radiopacity of filling materials. However, in some studies, indirect method by converting the radiographs to digital images were also used instead of optical densitometer [8].

Rasimick et al. stated that the imaging technique could affect the measured radiopacity values of the materials. Barium containing materials could have different radiopacities on film and phosphor store plates. Also differences could be found in the aluminum alloy of the step-wedge, exposure time, focal film distance, kVp, and mAs affects the radiopacity measurements of materials in situ [10].

The radiopacity of root canal filling should be at least 6 mm Al ,but excessive radiopacity of the material is not mentioned by ISO standardization, The radioopacity rates of Nano calcium carbonate polyurethane used in the present study was consistent with ISO standards. The inorganic fillers like Nano calcium carbonate, zinc oxide, calcium hydroxide in addition to barium sulfate are considered a radiopaque fillers, so they give the radiopacity for thermoplastic polyurethane base (TPU) which is a radiolucent material and corresponding to 1 mm of the Aluminum step wedge. As a conclusion for the present study, the Nano calcium carbonate polyurethane is a promising root canal filling material with good physical properties that comply with ISO standardization.

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پوختە

کەرەستا نانو کالسیوم کاربونات –بولی یوریسین لسەر بنیاتا کالسیوم کاربونات : ئامادەکرنا کەرەستێ وەک فلینک بو پرکرنا رهێت ددانا و دیارکرنا هندەک خاسلەتێت وێ ێت فیزیایی .

ئارمانج: ئارمج ژ فێ توێژینەوێ ئەو بوێ ئامادەکرنا کەرەستەکا نی ژبو پرکرنا رهێت ددانا کو نافێ وێ نانو کالسیوم کاربونات و پاش دیارکرنا سێ ج خاسلەتێت وێ ێت فیزیایی کو ئەو دهێتە حەلاندن و مژین و هەر هوسا رەشاتیا تیشکێ ێت هەین .

رێکا توێژینەوێ : کەرەستا بولی کاربونات و 1و4 هیکسامیسیلین دای ایزوسیانید برێژەیا 49.79 ٪ و کەرەستا بیوتان دیول هاتنە تێکەل کرن ژ بو دروست کردنا کەرەستا بولی یوریسین ئەوا سەر ینیاتا کەرەستا بولی کاربونات.هەر هوسا هندەک توزێت دیارکری هاتنە زیدەکرن لسەر کەرەستا بولی بولی یوریسین وەک توزا نانو کالسیوم کاربونات , اوکسید الزنک , هایروکساید کالسیوم , و کبریتات باریوم وب هندەک رێژیت ژێک جوودا یو دروست کرنا کەرەستا بولی یوریسین وەک کەرەستا برکرنا رهێت ددانا .

و هندەک تێستێت دیاری کری هاتنە ئەنجام دام ژبو هەلسەنگاندنا هندەک خاسلەتێت فیزیایی ێت فێ کەرەستا نی .

ئەنجام و پوختە : ئەوە کو کەرەستا کالسیوم کاربونات بولی یوریسین کەرەستەکا هاندەرە کو بکار بهێت ژ بو پرکرنا رهێت ددانا و خەسلەتێت وێ ێت حەلاندن و مژین و هەر هوسا رەشاتیا تیشکێ گەل پیفەرێت ISOێت نیف دەولەتی د گون

الخلاصة

مادة النانو كالسيوم كاربونات –البولي يوريثين المستند على الكالسيوم كاربونات : تحضير المادة كحشوة لملىء قناة جذور الاسنان وتقييم بعض الخصائص الفيزيائية

الاهداف: كان الهدف من هذه الدراسة هو تحضير مادة جديدة لحشو قناة جذر السن تسمى نانو كالسيوم كاربونات ومن ثم تقييم ثلاثة من الخصائص الفيزيائية لهذه المادة وهي قابلية الذوبان و الامتصاص والعتامة االشعاعية.

طريقة البحث : تم مزج مادة البولي كاربونات و 1و4 هيكساميثيلين داي ايزوسيانيد بتركيز 49;79 ٪ و مادة البيوتان ديول لتشكيل مادة البولي يوريثين المستندة على مادة البولي كاربونات.تم اضافة مساحيق معينة الى مادة البولي البولي يوريثين مثل مسحوق نانو كالسيوم كاربونات , اوكسيد الزنك , هايروكساید الكالسيوم , و كبريتات الباريوم وبنسب مختلفة لتشكيل مادة البولي يوريثين كما دة لحشو قناة جذر السن.وقد تم القيام باختبارات معينة لتقييم بعض الخصائص الفيزيائية لهذه المادة الجديدة

النتائج والخلاصة : ان مادة الكالسيوم كاربونات البولي يوريثين هي مادة مشجعة لا ستعمال كحشوة لجذور الاسنان وقابلية هذه المادة للذوبان والامتصاص والعتامة الشعاعية تتماشى مع المعايير العالمية ISO.

AN EFFICIENT METHOD FOR ISOLATION, CHARACTERIZATION AND IMMUNOPHENOTYPIC ANALYSIS OF HUMAN UMBILICAL CORD BLOOD DERIVED MESENCHYMAL STEM CELLS *IN VITRO*

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ABSTRACT

Background and Objective Umbilical cord blood (UCB) is an interesting source of mesenchymal stem cells (MSCs) for gene therapy, cell transplantation and cell therapy. This study is aimed to isolate, characterize and immunophenotypic analysis of human UCB- derived MSCs by using a simplified technique.
Methods Cord blood was collected after normal delivery of placenta by puncturing umbilical cord veins. The mononucleated cells (MNCs) was isolated after gradient centrifugation and cultured in Iscove,s Modified Dulbecco Medium (IMDM) supplemented with 10% Fetal calf serum (FCS). Cultures were maintained at 37C°,5% CO2 for two weeks. Then immunophenotypic analysis which was performed with CD90, CD71, CD34 and HLA-DR.
Results: The MSCs derived from MNCs appeared like the fibroblast cell and these cells were extensively expanded in culture. Immunocytochemistry staining indicated that UCB-derived MSCs were positive for CD90, CD71 and negative for CD34, HLA-DR. Conclusion: The result of the present study indicate that using this method can result in isolation of homogenous population of UCB- MSCs.

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Key words: Umbilical cord blood, mesenchymal stem cells,immunocytochemistry

Cord blood (CB) is the blood remaining in the umbilical cord and placenta after birth. When CB was unknown it was considered as a useless thing and normally discarded after birth, but with increasing knowledge and awareness to the benefits of this blood and after many clinical and individual evidences, CB is considered to be very important and useful blood so the people are saving or donating this blood to a CB bank [1]. Cord blood is known to contain both of hematopoietic stem cells (HSCs) and pluripotent mesenchymal stem cells (MSCs) [2].

Mesenchymal stem cells comprise a rare population of multipotent progenitors capable of supporting hematopoiesis and differentiation into various lineage including bone, muscle, brain, lung, heart, cartilage and variety of other connective tissues [3,4]. In their undifferentiated state, MSCs are spindle shaped and resemble fibroblastoid morphology [5]. Unlike HSCs, Fluorescence activated cell sorter (FACS) results showed that MSCs did not express antigens (CD11a, CD11b, CD14, CD34, CD45, HLA – DR and α – smooth actine), while express (CD29, CD44, CD71, CD 90, CD106, CD120a, CD124, HLA – ABC, SH2, and SH3) antigens, and they produce fibronectin, laminin and vimentin [6, 7].

Wang et al., [8] showed that MSCs from UCB when expanded in culture express adhesion molecules (CD44 and CD105), intigrin markers (CD29 and CD51) and MSCs markers (SH2 and SH3), but not express the markers of hematopoietic differentiation (CD34 and CD45).

In 2003 an attempt was made to isolate

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MSCs from the UCB using standard methodological approaches: the routine isolation and culture of isolated cells under conditions appropriate for BM– derived MSCs, these results suggest that CB contains a high number of MSCs – like elements forming colonies of fibroblastoid cells that may be successfully expanded in culture [3]. Then different techniques can be used to obtain MSCs such as plastic adhesion and negative selection (CD45, Gly-A) or positive selection (CD49-a, Stro-1, and CD133) with derived pattern identical to that of human BM-derived MSCs [4]. Therefore, UCB could be regarded as an alternative source of MSCs for experimental and clinical needs. The present study was done to isolate and detect the immunophenotypic analysis of human UCB- derived MSCs by using a simplified technique.

METHODS

Cord blood cell separation

Cord blood samples were collected freshly from discarded placenta of full term normal deliveries in Al- Kadhemia Hospital in Baghdad.

Blood (30 ml) was kept in anticoagulant treated tubes and used within10 hours after collection. Cord blood was diluted 1:1 with phosphate buffer saline (PBS), the diluted blood was carefully overlaid on Ficoll-paque at a ratio of 3:1 in 10 ml sterile conical tubes, the specimens were centrifuged on a cooling centrifuge for 25 minutes at 2000 rpm at 4°C.

After centrifugation, the MNC layer was recovered from the gradient interface and washed two to three times with PBS at 2000 rpm for 10 minutes at 4°C. The MNCs were cultured in sterile tissue culture flask (25ml) in 5ml of Iscove,s Modified Dulbecco Medium (IMDM) (Sigma) supplemented with 10% Fetal calf serum (FCS) (Sigma) and 100 units\ml Penicillin, and 100µg\ml Streptomycin. at final concentration 1-2 X 106 cells/ml. Cultures were maintained at 37 ºC in humidified atmosphere containing 5% CO2, with 50% of the media being changed every week according to previous studies (9,10). Cultures were screened continuously to get hold of developing colonies of adherent MSCs.

Fibroblastoid cells were recovered between 6-8 days after initial plating using 0.25% trypsin-EDTA and placed at a ratio of 1:3 in the same conditions for two weeks.

Immunophenotypic analysis of mesenchymal stem cells

After two weeks the fibroblastoid cells were dispersed with trypsin-EDTA and re-cultured in multi –well tissue culture plates (4-wells) at a density of 1X104 cells/well in IMDM supplemented with 10% FCS.

The cells were allowed to developing a monolayer of adherent cells within 4-5 days, then the medium was aspirated and the monolayer of adherent cells were washed two times with PBS. After that the monolayer was fixed with 4% paraformaldehyde diluted in PBS for 10 minutes, then detected by immunophenotypic procedure (11), which was performed with anti-rat and human body CD90, anti- human antibody CD71 (specific markers for detection of MSCs), anti- human antibody CD34 and HLA-DR anti- human antibody (specific markers for detection of HSCs).

The first step in immunophenotypic procedure was the addition of 4% hydrogen peroxide for 15 min. The second step was the addition of primary antibody (anti CD90,anti CD71,anti CD34 and HLA-DR)for 1h.except for the incubation period of CD90 which was over night, then the addition of secondary antibody (biotin) for 1h.The streptavidin conjugated to horse radish peroxidase was added to the wells for 1h.

The wells in all the above steps were incubated in a humidified chamber at 37C° and were washed extensively with PBS after each step.

For visualization of the stain, liquid DAB chromogen solution was added to the wells

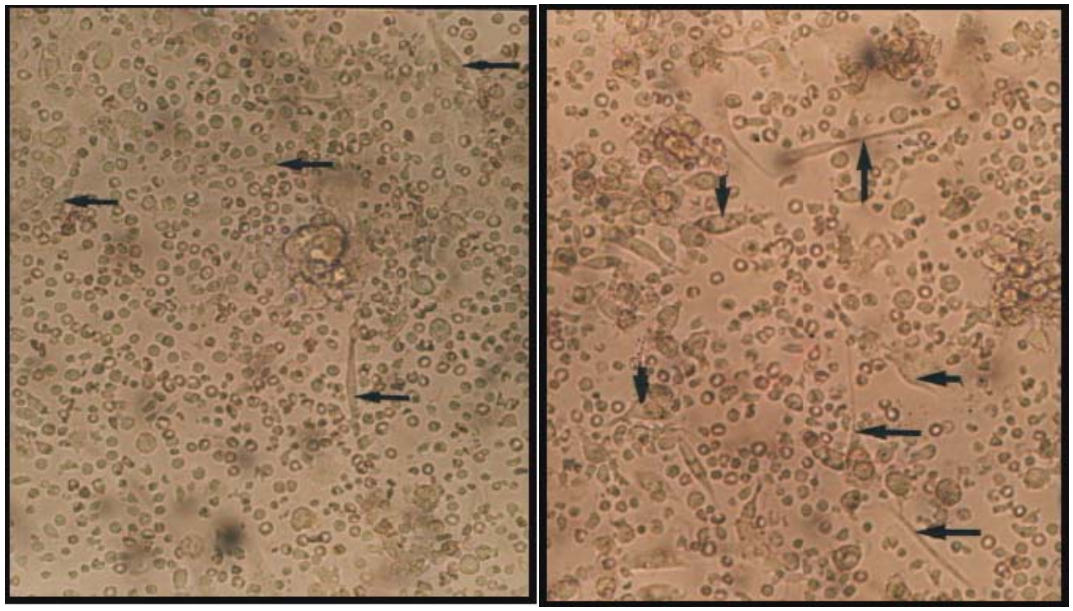
for 15 min then washed with PBS and counterstained in Harris hematoxylin for 2-3 min, washed with DW and then with PBS. The wells were mounted with glycerol and they were inspected by light microscope and photographed.

RESULTS

Culturing and propagation of umbilical cord blood-derived mesenchymal stem cells

After plating the MNCs, only a few cells were attached to the plastic culture flasks sparsely, and formed adherent cells while the non-adherent cells were discarded by the first medium change. The adherent cells began to proliferate, as soon as 5days after cultivation numerous fibroblast like-cells could be observed. These cells gradually grow to form small individual colonies displaying fibroblast-like morphology with short and long processes as well as, a small round cells with a high nuclear to cytoplasmic ratio can also be seen (Figures 1A,B).

Mesenchymal stem cells are characterized by their ability to aggregate and to form colonies comprising spindle-shaped cells deriving from a single cell. The number of cellular colonies with different sizes has obviously increased. In large colonies, cells were more densely distributed and shown spindle shaped. These colonies are termed colony forming unite derived fibroblast like cells (CFU-F) and usually used as an indicator for mesenchymal progenitor potential. These colonies gradually expanded in size and interconnected with adjacent colonies (Figures 2 A, B). When the cells grew to 80% confluence, the cells were ready for first passage. So, after first passage, the MSCs began to grow and formed colonies and by the end of the second week, a homogeneous layer of fibroblastoid-like cells occupied the whole plastic surface (Figures 3 A,B). So according to this result and to the morphological aspect and growth characterization of these MSCs, they are considered to be fibroblastic F-type cells.



A B

Figure 1. UCB-derived MSCs cultured in IMDM+10%FCS. (A): After 3 days, the adherent cells began to proliferate and have a fibroblast-like morphology (arrows) (X100.8). (B): After 5 days, the MSCs appeared with long (indicating long arrows) and short (indicating short arrows) processes as well as a small round cells can also seen (X160).

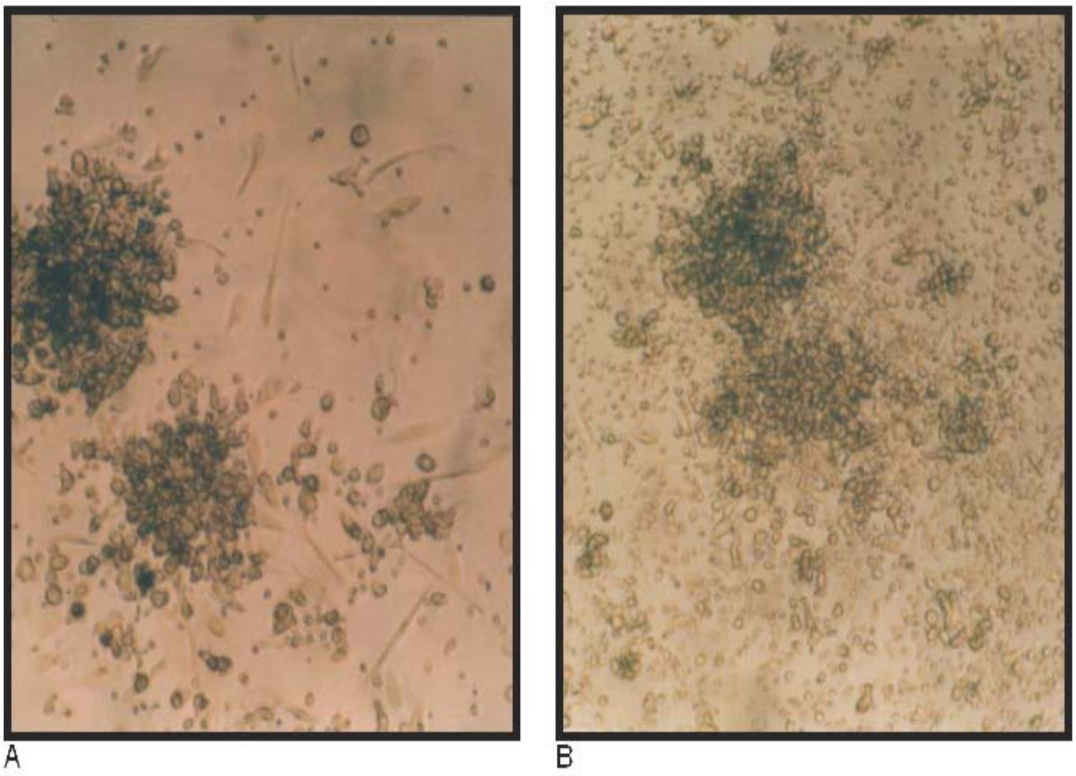


Figure 2. Primary culture of UCB-derived MSCs cultured in IMDM+10% FCS. (A): the appearance and growth of MSCs colonies after 7 days (X100.8). (B): note that the colonies interconnected with each other and reaching a confluent stage so they were ready for passaged(40x).

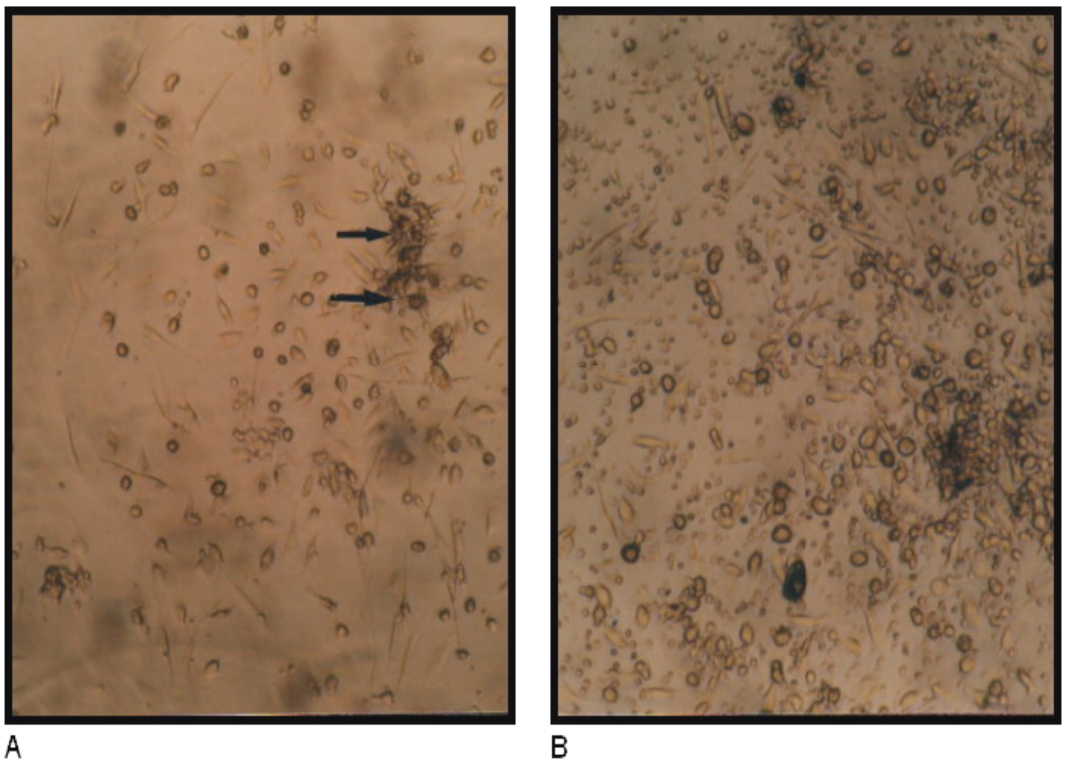


Figure3. The first passage culture of UCB-derived MSCs. (A): 3 days after reseeding, the cells began to proliferate and formed colonies (arrows) (X40). (B): 7 days after reseeding, the cells were expanded and occupied the whole plastic surface (X100.8).

The cells were reseeded in same conditions for the second passage culture. These adherent cells could be readily expanded in vitro by successive cycles of trypsinization, seeding and culture every 5 days without visible morphologic alteration.Immunophenotypic characterization of umbilical cord blood-derived mesenchymal stem cells.

The results of the Immunophenotypic study revealed that MSCs were stained positive for CD71+ and CD90+ (Fig. 4 A,B), while negative for CD34- and HLA-DR- (Figure 4 C,D), indicating that these cells were not of hematopoietic origin.

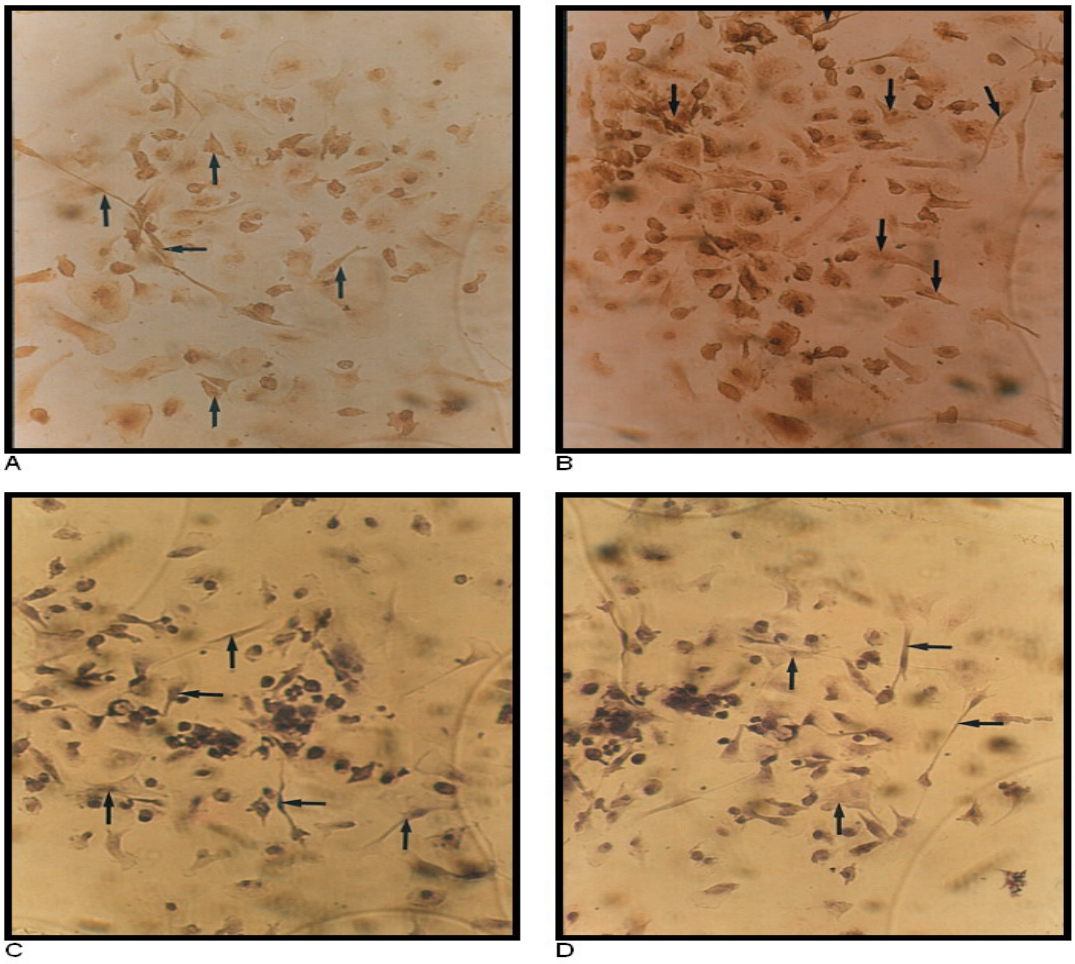


Figure 4. Immunophenotypic analysis of UCB-derived MSCs at the 2nd passage, the positive cells were stained with brown color (DAB stain), while the negative cells were stained with blue color (hematoxylin stain). (A): MSCs show positive response for CD71 (arrows) (X100.8). (B): MSCs show positive response for CD90 (arrows) (X100.8). (C): MSCs show negative response for CD34 (arrows) (X100.8). (D): MSCs show negative response for HLA-DR (arrows) (X100.8).

DISCUSSION

The present study, aimed to isolate and expanded UCB-derived MSCs in culture by using a simple culture technique. Although the evidence for the isolation of fibroblastoid cells with MSC characteristics from UCB is conflicting. But the results of the present study reveled that MSCs were isolated from full-term UCB with efficiency of greater than 50%. This good percentage is probably due to the use of IMDM, a medium which as indicated by others [12], represents the perfect culture medium for isolation and maintaining MSCs in vitro. Besides, it is known that FCS is crucial for the growth of MSCs [13], so the addition of FCS to the

culture medium might provide additional growth and adherence factors, favoring the generation of MSCs from UCB.

Different methods were applied for obtaining MSCs from UCB. The first method was the classic plastic adhesion and sub cultures, the second method was the using of 5% from condition medium.

The third method was the immunoselection for specific CD markers of MSCs directly from fresh samples [4]. In the present study, the classic plastic adhesion method was used for obtaining a plate-adhering population from plating total MNCs, raising the possibility that such heterogeneous population may contain progenitors of all three germ layers.

Tasi et al., [14] reported that under routine culture conditions for MSCs, UCB-derived MNCs can be divided into two major categories: adhering and non – adhering cells. Furthermore, MSCs can also be classified according to their morphological aspects and growth characteristics into two groups: epitheloid (E-type) cells, and fibroblastic (F-type cells), so from the result of the present study and according to the morphological aspects and growth characterization of MSCs, they are considered to be fibroblastic F-type cells. This finding are in agreement with the results described by others [7,15,16,17,] who demonstrated that freshly isolated cells principally displayed a fibroblast-like appearance in the first week and during the second week, they typically appeared as slender cells with a narrow cytoplasm. Then after 12-14 days, they grew to 100% confluence. These attached MSCs with fibroblastic phenotype, having a fairly uniform morphology that is similar to that of MSCs isolated from BM [18].

Immunophenotypic characterization of umbilical cord blood-derived mesenchymal stem cells

The result of morphological studies and immunophenotyping of cultured MSC-like cells from human UCB suggest that these cells closely resemble cultured BM-MSCs obtained by other studies [3, 19].

The present result indicated that the UCB-derived MSCs are positive for CD71+, CD90+ while negative for CD34-, HLA-DR-, a finding that is compatible with that of other authors [7,14,17,20,21], who demonstrated that UCB-derived MSCs have a characteristic set of surface markers that include cluster of differentiation (CD) markers, for example MSCs are positive for: CD29, CD44, CD71, CD90, HLA-A, B, C and SH2, SH3, and negative for: CD10, CD11b, CD14, CD34, CD45, CD117 and HLA-DR, DQ.

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پوختە

ڕێگەیەکی بەهێز بۆ جیاکردنەوە, وەسف و شیکردنەوەو دیاری کردنی جۆری بەرگری کە لە خوێنی ناوکە پەتکی مرۆڤ وەرگیراون لە دەرەوەی لەش

باکگراوند و مەبەستی لێکۆڵینەوە:خوێنی ناوکە پەتك بە سەرچاوەیەکی خانە دروستکەرە گۆشتیەکان دادەنرێت, کە بەکاردێت بۆ چارەسەری جینی , خانەیی و گواستنەوەی خانەکان. ئامانج لەم لێکۆڵینەوەیە بریتییە لە جیاکردنەوەو وەسف کردنی بۆ شیکردنەوەی بەرگری ڕووکەشی خانە دروستکەرەکانی کە لە خوێنی ناوکە پەتك دەرچوون, ئەمەش بە پشت بەستن بە تەکنیکی سادەدە.

ڕێگای لێکۆڵینەوەکە: نموونەی خوێنی ناوکە پەتك لە وێلاشی دوای لەدایك بوونی سروشتی وەرگیراوە بەکونکردنی خوێنهێنەری ناوکە پەتك و خانە تاك ناوکەکان جیاکرایەوە بەبەکارهێنانی سەنترفیوجی پلەبەندی , دواتر خانە تاك ناوکەکان لەسەر میدیای گەشەیی (IMDM) و10٪ سیرەمی گۆلکی بۆزیادکرا, دواتر ئەم کەلچەرانە لەژێر پلەی 37سo و 5٪ گازی دووەم ئۆکسیدی کاربۆن گەشەی پێکرا. دواتر شیکردنەوەی بەرگری ڕووکەشی بۆ ئەم خانانە ئەنجام درا بەبەکارهێنانی (CD90, CD71, CD34وHLA-DR).

ئەنجام: خانە دروستکەرە گۆشتیەکان بەشێوەی خانە دروستکەرەفایبەرییەکان دەرکەوتن وە ئەم خانانە توانای فراوان بوونێکی گەورەیان هەبوو لەم میدیا گەشەییانە. دەرئەنجامی شیکردنەوەی ڕووکەشە خانە دروستکەرەکان دەریانخست کە خانە دروستکەرە گۆشتیەکان وەرگیراوە لە خوێنی ناوکە پەتك بە وەلامدانەوەی پۆزەتیڤ بۆ (CD90 وCD71) وە وەلامدانەوەی نێگەتیڤ بۆ (CD34 و HLA-DR).

دەرئەنجام: ئەنجامەکانی ئەم لێکۆڵینەوەیە بەبەکارهێنانی ئەم ڕێگایە دەریخست کە دەتوانرێت خانە دروستکەرە گۆشتیەکان چونیەك جیابکرێتەوە لە خوێنی ناوکە پەتك.

الخلاصة

طريقة كفوءة لعزل، ووصف ولتحليل المظهر المناعي للخلايا الجذعية اللحمية المشتقة من دم الحبل السري خارج الجسم الحي

خلفية وهدف البحث: يعد دم الحبل السري مصدرا"للخلايا الجذعية اللحمية, والتي تستخدم في كل من العلاج الجيني والخلوي والنقل الخلوي. تهدف هذه الدراسة إلى عزل ووصف والى التحليل المناعي المظهري للخلايا الجذعية المشتقة من دم الحبل السري للإنسان, وذلك باعتماد تكنيك مبسط.

طريقة الدراسة:جمعت عينات دم الحبل السري من المشيمة بعد الولادات الطبيعية بثقب أوردة الحبل السري وعزلت الخلايا الاحادية النواة بأستخدام النبذ المركزي المتدرج الكثافة , وزرعت الخلايا الاحادية النواة في وسط زرعي (IMDM)مضاف اليه 10٪ مصل جنين العجل وحضنت المزارع لمدة إسبوعين بدرجة حرارية 37مْ و5٪ غاز ثنائي اوكسيد الكاربون. ومن ثم إجراء التحليل المناعي المظهري لهذه الخلايا باستخدام الواسمات التالية: .(CD90, (CD71, CD34 and HLA-DR)

النتائج: ظهرت الخلايا الجذعية اللحمية بشكل شبيه بالارومات الليفية وهذه الخلايا لها القدرة على التوسع بشكل كبير في الوسط الزرعي. أظهرت نتائج التحليل المظهري المناعي ان الخلايا الجذعية اللحمية المشتقة من دم الحبل السري ذات استجابة موجبة (CD71,CD90) وذات استجابة سالبة (CD34,HLA-DR).

الاستنتاج: تشير نتائج الدراسة الحالية باستعمال هذه الطريقة ممكن عزل خلايا جذعية لحمية متجانسة من دم الحبل السري.

USE OF THE D-TEST METHOD FOR DETECTION OF INDUCIBLE CLINDAMYCIN RESISTANCE IN *STAPHYLOCOCCI* ISOLATES

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ABSTRACT

Background and objective: The resistance to antimicrobial agents among Staphylococci is an increasing problem. This has led to renewed interest in the usage of Macrolide-Lincosamide-Streptogramin B (MLSB) antibiotics to treat *Staphylococcus aureus* infections. Clindamycin resistance in *Staphylococcus* species can be either constitutive or inducible which can lead to therapeutic failure. In vitro routine tests for clindamycin susceptibility may fail to detect inducible clindamycin resistance thus necessitating the need to detect such resistance by a simple D test on routine basis.
Methods: Between October 2009-March 2010, 36 isolates *of Staphylococci* spp. were recovered from 130 urine samples of children with UTI at Rapareen Pediatric Hospital in Erbil city. All the *Staphylococca*l spp. were identified by using standard microbiological procedures [4]. All isolates were tested for inducible clindamycin resistance by the D-zone test.
Results: Out of 36 isolates of *Staphylococc*i spp.16 isolates were Staphylococcus aureus (56.3% MRSA, 43.8% MSSA) and 20 were coagulase negative S*taphylococci*. Inducible clindamycin resistance was demonstrated in 33.3% of methicillin resistance *Staphylococcus aureus* (MRSA), 14.3% in methicillin sensitive *Staphylococcus aureus* (MSSA) and 35% in coagulase negative *Staphylococci.*
Conclusions: This study indicates importance of the D-zone test in detecting inducible clindamycin resistance in *staphylococci* to aid in the optimal treatment of patients.

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Key words: Staphylococcus, inducible clindamycin resistance, D-test

Staphylococcus aureus and coagulase negative *Staphylococci* are recognizes as causing nosocomial and community acquired infections. Macrolid (e.g., erythromycin), lincosamide (e.g., clindamycin) and streptogramin B (e.g. quinupristin–dalfoprin) antimicrobial agents are widely used in the treatment of Staphylococcal infection[1]. Macrolid antibiotics are bacteriostatic agents that inhibit protein synthesis by binding reversibly to 50s ribosomal subunits of susceptible organism [2].

The Macrolid, lincosamide and streptogramin B (MLSB) family of antibiotics has three different mechanisms of resistance–target site modification, enzymatic antibiotic inactivation and macrolide efflux pumps. Clindamycin, a lincosamide antibiotic, is among the limited choice of antimicrobials effective against MRSA. There is concern about use of this antibiotic in the presence of Erythromycin resistance because of the possibility of induction of cross- resistance among members of the macrolide, lincosamide, streptogramin B group. As MRSA infections have become increasingly common in the community setting,the development of empirical antimicrobial therapeutic strategies for Staphylococcal infections has become more problematic .Clindamycin has long been an option for treating both MSSA and MRSA infections. However, expression of inducible resistance to clindamycin could limit the effectiveness of this drug [3]. Previous reports indicated that treatment of patients harboring iMLSB resistant S. aureus with clindamycin might lead to development of c MLSB resistant strains and subsequently, therapeutic failure.

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Unfortunately, the iMLSB phenotype cannot be recognized by using standard susceptibility test and require specific methods. A test known as disk approximation test or simply D-test detects MLSB resistance pattern of Staphylococci [4].

METHODS

Between of October 2009-March 2010, 36 isolates of Staphylococci spp. were recovered from 130 urine samples of children with UTI at Rapareen Pediatric Hospital in Erbil city. Pathogens were isolated using standard media, including CLED agar, Blood agar, Mannitol salt agar, DNase agar and specimen were inoculated using standard techniques. Plates were incubated at 37c˚for overnight. Identification of all isolates was done on the basis of biochemical tests using catalase, ,coagulase, DNase tests and API STAPH. Methicillin resistance was detected by using oxacillin (OX) (1µg), cefoxtitin(Cn) (30 µg) and methicillin (ME) (5 µg) disks were placed on the inoculated Mueller Hinton agar plates, then incubated at 35c˚ for 24 hours. The diameter of inhibition zones were measured to the nearest millimeter. An isolates was considered to be MRSA strain if the cefoxtitin, methicillin and Oxacillin inhibition zones diameter were ≤14, ≤9 and ≤13 respectively. To identify the MLSBi phenotype, the D-test was performed; suspension equivalent to 0.5 McFarland of each freshly cultured isolate in normal saline was prepared and inoculated onto a Mueller–Hinton agar plate and discs of clindamycin (CL)(2µg) and erythromycin (ER) (15µg) were placed at a distance of 12mm (edge to edge). Plates were incubated at 37° C for 24 hr. The diameter of inhibition zone was measured; in addition, each CL zone was examined carefully to detect a flattening or blunting of the shape (D-shape zone) near E disc. The disc diffusion test, based on the D test, four phenotypes were interpreted as follows:

1-D test Positive (iMLSB Phenotype): Inducible resistance to Clindamycin was manifested by flattening or blunting of the CL zone adjacent to the ER disc, giving a D shape (D-test positive).
2-Sensitive phenotype (MS): sensitive to both clindamycin and erythromycin.
3-Constitutive Phenotype (cMLSB): Resistant to both ER and CL.
4-D test Negative (MLSB Phenotype): No flattening of the CL zone; Resistant to ER but susceptible to CL. Staph. aureus ATCC 25923 was used for quality control of clindamycin and erythromycin disks according to the standard disk diffusion procedure.

Statistical Analysis: Data analysis and calculate of P-value was done using SSPS version 16.0 through contingency coefficient test (CC test). A P-value≤ 0.05 is regarded as significant (S).

RESULTS

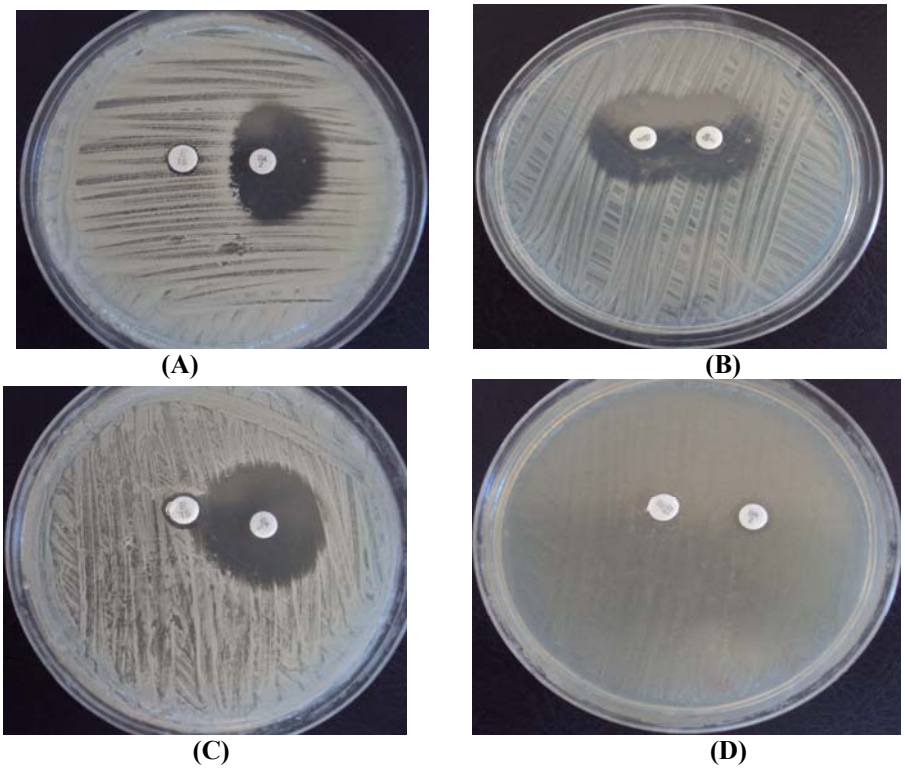
Out of 36 isolates of *Staphylococci* spp.16 isolates were Staphylococcus aureus (56.3% MRSA, 43.8% MSSA) and 20 were coagulase negative Staphylococci. Inducible clindamycin resistance (iMLSB) was demonstrated in 30.6 of *Staphylococci* isolates (33.3% in methicillin resistance Staphylococcus aureus (MRSA), 14.3% in methicillin sensitive *Staphylococcus aureus* (MSSA) and 35% in coagulase negative Staphylococci). While 36% of isolates showed D-test negative (MLSB) and was found in 14.3% of MSSA, 60% of coagulase negative *Staphylococci* but not detected in MRSA (0%). Constitutive Phenotype (cMLSB) was detected in 5.6% of isolates in which 11.1% MRSA, 5% coagulase negative *Staphylococci* whereas cMLSB was not found in MSSA and 27.6% of isolates had sensitive phenotype (MS) which was detected in 55.6% MRSA and 71.4% MSSA, but not found in coagulase negative *Staphylococci* (Table 1). (Figure 1) shows iMLSB phenotype in MRSA. (Figure 2) Disc diffusion test for inducible clindamycin resistance which shows the four phenotypes in the *Staphylococci* isolates.

Table 1. Distribution of *Staphylococcal* isolates according to phenotypes.

Staphylococci isolates	No. (%) of isolates	Phenotypes (iMLS$_B$) D-test positive No. (%)	(MLS$_B$) D-test negative No. (%)	(cMLS$_B$) Constitutive Phenotype No. (%)	Sensitive phenotype (MS) No. (%)
MRSA	9 (25%)	3 (33.3%)	0 (0%)	1 (11.1%)	5 (55.6%)
MSSA	7 (19.4%)	1 (14.3%)	1 (14.3%)	0 (0%)	5 (71.4%)
Coagulas negative *Staphylococci*	20(55.5%)	7 (35%)	12 (60%)	1 (5%)	0 (0%)
Total	36 (100%)	11(30.6%)	13 (36.0%)	2 (5.6%)	10 (27.8%)

P- value =0.002 HS

Figure 1. Inducible clindamycin resistance in MRSA (D- test positive).



(A) **(B)**

(C) **(D)**

Figure 2. Disc diffusion test for inducible clindamycin resistance showing four phenotypes: (A) D–test positive phenotype; (B) Sensitive phenotype (MS); (C) D–test negative phenotype; (D) Constitutive Phenotype.

DISCUSSION

The present study showed that inducible clindamycin resistance (iMLSB), constitutive (cMLSB), sensitive (MS) and D-test negative MLSB were demonstrated in 30.6%, 5.6%, 27.8%, and 36% respectively. These results agree with results obtained by Abdulrahman[5] who showed that out Of the 291 ER Staphylococci studied, 38.8% were iMLSB, 28% were cMLSB, and 33% of isolates were remained susceptible clindamycin (MS).

Our study showed that inducible clindamycin resistance was found in 33.3% MRSA, 14.3% and 3.5% of coagulae negative *Staphylococci* isolates. In a study conducted in India, Ciraj et al[6] reported that inducible clindamycin resistance was found in 38.4% of MRSA, 12.9% of MSSA and 6.3% of coagulae negative *Staphylococci*. Deotale et al[7] reported that 14.5% of isolates showed iMLSB, 3.6% showed cMLSB while remaining 14.1% showed MS phenotype and they added that iMLSB and MS phenotype were found to be higher in MRSA as compared to MSSA (27.6%, 24.3% and 1.6%, 4% respectively). Shantala et al[8] stated that out of 230 *Staphylococci* isolates, 24.78% were found to be iMLSB phenotype and they demonstrated that 32.5% of MRSA isolates and 15.53% of MSSA isolates showed iMLSB resistance and 25.39% of MRSA isolates and 9.6% of MSSA isolates showed cMLSB.

The present study showed that in 20 isolates of coagulase negative Staphylococci 35% had iMLSB, 5% had cMLSB, 60% D-test negative (MLSB) and no MS phenotype. Hamilton- Miller et al[9] determined that in coagulase negative *Staphylococci* strains 31% had iMLSB, 11% had cMLSB and 13% had MS. Abdulrahman5 reported that in coagulase negative *Staphylococci* strains 20.7% had iMLSB, 26% had cMLSB and 52.8% had D-test negative (MLSB).In a study conducted in Turkey, Yilmaz et al[10] showed that 24.3% were determined to have the inducible MLSB resistance phenotype and they added that when the *Staphylococcus aureus* and coagulase negative Staphylococci strains among all staphylococcal isolates were statistically compared, iMLSB in coagulase negative *Staphylococci* strains was determined to be 23% more positive.

Clindamycin is indicated for the treatment of soft tissue infections, pediatric infections caused by *Staphylococci*, or for patients allergic to β-lactam agents[11].

Clinically, bacterial strains exhibiting MLSB have a high rate of spontaneous mutation to constitutive resistance, and the use of non inducer antibiotics such as clindamycin can lead to the selection of constitutive mutants and may result in clindamycin treatment failure. The emergence of MDR *Staphylococci* has left very few therapeutic options for clinicians. A therapeutic decision is not possible without the relevant clinical and microbiological data. The increasing frequency of MRSA with in vitro inducible clindamycin resistance raises a concern of clindamycin treatment failures[3].

In vitro susceptibility testing for clindamycin may indicate false susceptibility by the broth microdilution method and by disk diffusion testing with erythromycin and clindamycin disks in nonadjacent positions. The true sensitivity to clindamycin can only be judged after performing D test on the erythromycin resistant isolates[12].

CONCLUSIONS

The implementation of the D-test a simple, auxiliary method with routine antibiotic susceptibility testing, delineates inducible and constitutive clindamycin resistance. The high rates of occurrence of inducible resistance in the MRSA, MSSA and CoNS strains raise concerns that clindamycin treatment failures may occur with MSSA and coagulase negative

Staphylococci as well as with MRSA infection. Consequently early detection helps in the use of clindamycin only in infections caused by truly clindamycin susceptible *Staphylococcus aureus* and thus helps to avoid treatment failures.

RECOMMENDATIONS

We recommend that microbiology laboratories perform erythromycin induction test on all ER-R CL-S staphylococcal isolates prior to reporting clindamycin susceptibility

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پوخته

به کارهێناني ڕێگای D- test بو ده ست نیشانکردنی به رگری هاندراو بۆ کلیندە مایسین لە جیاکەرەوەکانی *Staphylococci*

پێشە کی وئارمانج:- بەرگری بۆ دژە زیندە گیەکان لە بەکتریا *Staphylococci* کێشە یە کە ڕوو لە زیاد بووندایە. ئە وە دە بێتە هۆی دووبارە چاو پێخشاندن و گرنگی دان بە بە کار هێنانی دژە زیندە گیەکانی Macrolide-Lincosamide-Streptogramin B(MLS_B) لە چارە سە رکردنی تووشبوون بە *Staphylococcus aureus*. بە رگری بۆ کلیندە مایسین لە *Staphylococci* لە وانە یە جێگیر یان هاندراو بێت کە دە بێتە هۆی سە رنە کە وێتنی چارە سە ر. تاقیگردنەوە ی هە ستیاری ڕۆتینی بۆ کلیندە مایسین لە وانەیە سە رکە وتوونە بێت لە دە ست نیشانکردنی بە رگری هاندراو بۆ کلیندە مایسین بۆیە بێوستە ئە م بە رگرە بدۆزرێتە وە بە بە کارهێناني ڕێگای D-zone test.

ڕێکێن ڤە کولینێ:- ئە م توێژینە وە یە ئە نجام دراوە لە نە خوشخانە ی ڕاپە رینی منداڵان لە شاری هە ولێر، لە مانگی تشرینی یە کە می 2009 تا مانگی ئاداری 2010, 36 *Staphylococci spp.* جیاکراوە لە 130 نموونە ی میزی منداڵان تووشبو بە هە وکردنی ڕێرەوە کانی میز, هە موو جیاکراوەکان شێکرانەوە بە ڕێگای تاقیکراوەنەوە کێلگەیی کیمیای ژیانی ستاندارو،هەروە ها هە موو جیاکراوەکان تاقیکراوەنەوە بۆ دە ست نیشانکردنی بە رگری هاندراو بۆ کلیندە مایسین بە بە کارهیناني ڕێگای D- zone test.

ئە نجام:- لە کۆی 36 جیاکراوەی *Staphylococci*، 16 *Staphylococcus aureus* (56.3% MRSA, 43.8% MSSA) وە 20 *Staphylococci* coagulase negative بوو. بە بە کار هێنانی ڕێگەی D-zone test بۆ تاقیکردنەوە ی ڕێژەی بەرگری هاندراو بۆ کلیندەمایسین لە *Staphylococci* ، ئەنجامەکان دەریان خست توانای بەرگریکردن هاندراو بۆ کلیندە مایسین بینرا لە 33.3% MRSA، 14.3% MSSA وە 35% coagulase negative *Staphylococci*.

دە رئە نجام:- ئە م توێژینە وە یە بۆمان دە ردە خات گرینگی D- zone test لە دە ست نیشانکردنی بە رگری هانداراو بۆکلیندەمایسین لە جیاکەرەوەکانی *Staphylococci* بە مە بە ستی بە کار هێنانی باشترین چارە سە ر بۆ نە خۆشە کان.

الخلاصة

استعمال طريقة D-test

لكشف عن المقاومة المحفزة لكليندمايسيين في العزلات المكورات العنقودية (*Staphylococci*)

خلفية واهداف البحث:- مقاومة المكورات العنقودية للمضادات الحيوية مشكلة متصاعدة مما يؤدي الى اعادة النظر في استعمال مجموعة المضادات الحيوية (MLS_B) Macrolide-Lincosamide-Streptogramin B في معالجة اصابات ببكتريا *Staphylococcus aureus* . ان المقاومة للمضاد الحيوي كليندمايسين في المكورات العنقودية اما تكون ثابتة او محفزة والتي قد يؤدي الى فشل العلاجي. في المختبرات المايكروبايولوجية اختبار الحساسية لكليندمايسيين بالطريقة الروتينية ربما قد تفشل في اكتشاف المقاومة المحفزة لكليندمايسيين ,لذا اوجب الحاجة لاكتشاف هذة المقاومة بأستخدام اختبار(D-zone test) .

طرق البحث:- اجريت هذه الدراسة في مستشفى رابرين للأطفال في مدينة اربيل, ما بين تشرين الاول 2009 واذار2010 . تم عزل 36 عزلة من المكورات العنقودية من130 عينة الادرار جمعت من الاطفال المصابين بالتهاب المجاري البولية. شخصت جميع عزلات المكورات العنقوديه بواسطة الاختبارات المايكروبايولوجية القياسية . اجريت الحساسيه للمضاد الحيوي (مقاومة المحفزة لكليندمايسيين) على البكتريا المعزولة بطريقة (D- zone test).

النتائج:- اظهرت النتائج ان من اصل 36 عزلة من المكورات العنقودية 16 عزلة كانت *Staphylococcus aureus* (56.3% MRSA, 43.8% MSSA) و 20عزلة كانت *coagulase negative Staphylococci*. وبينت النتائج ان 33.3% MRSA, 14.3% MSSA و 35% coagulase negative *Staphylococci* كانت موجبة لاختبار D-zone test (المقاومة المحفزة لكليندمايسيين).

الاستنتاجات:- اكدت الدراسة الحالية اهمية اختبار D- zone test في اكتشاف عن المقاومة المحفزة لكليندمايسيين في العزلات المكورات العنقودية بغية توصيف المعالجة المثلى للمرضى.

SERUM PROTEIN PENTOSIDINE LEVELS AMONG OFFSPRING OF INDIVIDUALS WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT

Background and objectives: The increase concentrations of serum protein pentosidine in diabetes are generally accepted to be a consequence of hyperglycemia. Offspring of individuals with type 2 diabetes mellitus are known to have increased risk for hyperglycemia state and metabolic abnormalities .Our aim was to determine serum pentosidine levels in subjects with positive family history of diabetes mellitus in an attempt to investigate its state towards risk of metabolic disease.

Methods: A case-control study design was conducted on 202 apparently healthy subjects (aged 20-40 years). Among these, 100 subjects (50 sons and 50 daughters) were offspring of individuals with type 2 diabetes mellitus. The remainders were 51 males and 51 females of comparable age and sex selected from the staff and students of Medical College. Inclusion criteria were negative family history of diabetes mellitus. At the baseline, the demographic data was collected, and then a blood sample was taken in fasting state to measure serum pentosidine, glucose, lipid profile and uric acid. Oral glucose tolerance test was performed. Insulin levels were measured and homeostatic model assessment-insulin resistance was calculated.

Results: Serum pentosidine levels were significantly higher in the subjects with positive family history of type 2 diabetes mellitus as compared to those with negative family history (serum pentosidine ng/ml, 33.8 Vs 19.4). There were positive relationship between serum pentosidine and metabolic risk factors. A highly significant correlation between serum pentosidine and insulin resistance was observed ($r=0.557$, $p<0.001$). Pentosidine determination appear to be a good screening method for risk evaluation of metabolic disease since it presents high specificity (94.1%) and sensitivity (54.0%) values with an area below the ROC curve =0.821,(95% CI 0.761-0.872). The positive and negative predictive value was 90 and 67 respectively.

Conclusion: We concluded that determination of serum pentosidine is a good screening method for metabolic risk stratification among offspring of individuals with type 2 diabetes mellitus

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Key words: Pentosidine, Diabetes Mellitus

Pentosidine is one of the few chemically characterized advanced glycation end products, is generated by non-enzymatic glycation and oxidation of protein.[1]The increase concentrations of serum protein pentosidine in diabetes are generally accepted to be a consequence of hyperglycemia.[2,3] Results from the Iraqi study which investigated Arab population, showed an impaired glucose tolerance (IGT) and previously undiagnosed diabetes (PU-DM) prevalence of 17.8% and 3.7%.4 In North Iraq, most recent study estimated that 14.3% and 10.9% of the Kurd population had IGT and PU- DM respectively .[5] Offspring of individuals with type 2 diabetes mellitus (type 2 DM) are known to have increased risk for hyperglycemia state and metabolic abnormalities.[6] Hence, it is essential to know the concentration of serum pentosidine in individuals at a high risk category for developing type 2 DM; i.e. first –degree relatives of type 2 DM and to investigate its state towards risk stratification of metabolic disease.

METHODS

This study included one hundred subjects with positive family history of type 2 DM. They were 50 sons and 50 daughters offspring of individuals who attended the Duhok Diabetes Center, who were

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diagnosed with type 2 DM ; and one hundred two subjects (51 males and 51 females) of comparable age and sex, who were with negative family history of diabetes served as a control group . A protocol involved that, all individuals diagnosed as type2 DM , randomly (every third) were interviewed and informed about the nature of the study and asked to bring their first degree relatives (sons or daughters) at the range of 20-40 years in fasting state for at least 12 hours. The study protocol was approved by the ethical committee of the General Directorate of Health in Duhok Governorate.

At the beginning, a total of 183 subjects were participated in the study. After exclusion of 83 respondents who were with a BMI>27 or <20 Kg/m2, non-fasting, as well as those who had an acute infection that required antibiotic therapy, the remainder were enrolled in the study. After obtaining the demographic data, fasting blood samples were obtained from the subjects and pentosidine, glucose, triglycerides, high density lipoprotein-cholesterol and uric acid levels were estimated. Oral glucose tolerance test was performed. Insulin levels were measured and homeostatic model assessment-insulin resistance was calculated according to the formula adopted by Esteghamati et al.[7] Monobind microplate ELISA test was used for the quantitative determination of pentosidine and insulin levels. Serum concentrations of glucose, lipid profile and uric were measured by clinical chemistry Auto Analyzer ,KENZA. TX .The blood pressure was measured in the right arm in supine position; 3 readings were taken and the mean value of the 3 readings was taken as the final recording. The waist circumference was measured using a plastic metric tape applied midway between the lower costal margin and the iliac crest. The offspring of individuals with type 2 DM were classified into two groups: metabolically obese normal weight (MONW) and non-metabolically obese normal weight (non-MONW) according to the established criteria for identifying an MONW individual.[8] Statistical analysis was done by using the SPSS software version 18.0.

RESULTS

The characteristics of the study subjects are presented in (Table 1). Substantial differences in most the variables examined were observed. Serum pentosidine levels were higher in subjects with positive family history of type 2 DM than in negative family history group at a cutoff value of 29 ng/ml , the prevalence of hyperpentosidinemia was 60% Vs 8.8% (Figure 1).There were positive relationships between serum pentosidine and metabolic risk factors (Table 2). A highly significant correlation between serum pentosidine and insulin resistance was observed ($r=0.557$, $p<0.001$.) , (Figure 2) .Pentosidine at a maximum cutoff value of 29 ng/ml appear to be a good screening method for risk evaluation of metabolic disease since it presents high specificity (94.1%) and sensitivity (54.0%), values with an area below the ROC curve =0.821,(95% CI 0.761-0.872). The positive and negative predictive value was 90 and 67, respectively. The extrapolation of data to a ROC curve is shown in (Figure 3). Subjects who classified with MONW had a higher pentosidine levels as compared with non-MONW group (Table 3).

DISCUSSION

This study has provided definitive evidence for the first time that offspring of individuals with type 2 diabetes mellitus had hyperpentosidinemia, but no evidence of Body Mass Index abnormality. These subjects had increased levels of insulin and had impaired insulin sensitivity and had a high prevalence of MONW as compared to those in the negative family history group. In prospective study of non- diabetic offspring of type 2 diabetes patients,

hyperinsulinemia, and impaired insulin sensitivity with increased levels of fructosamine and glycated hemoglobin (HbA1C) had been suggested.[9] Our data showed that pentosidine is related with majority of MONW parameters and a significant positive correlation between pentosidine and insulin resistance was observed.

Table 1. Subject characteristics

Variable	Positive family history of type 2 DM	Negative family history of type 2 DM	P-value
Number	100	102	
Age (years)*	25.0±5.1	24.4±3.1	NS
Male sex**	50(50.0)	51(50.0)	NS
Body mass index (kg/m^2)*	24.1±2.2	22.9±1.9	NS
Waist circumference (cm)*	80.4±10.5	75.1±10.3	NS
Hypertension** (BP>135/85 mm/Hg)	29(29.0)	4(3.9)	<0.001
Diabetes mellitus** FSG>126mg/dl)	10(10.0)	1(0.98)	<0.001
Impaired fasting glucose** (FSG 100-125 mg/dl)	14(14.0)	1(0.98)	<0.001
Dyslipidemia** (Serum triglycerides>150mg/dl+ HDL- ch<35 mg/dl)	33(33.0)	7(6.8)	<0.001
Hyperuricemia ** (Serum uric acid>7.0 mg/dl)	6(6.0)	2(1.96)	NS
Fasting Insulin(µIU/ml)*	8.4+2.1	4.9+2.6	<0.001
HOMA-IR *	2.0+0.7	1.1+0.6	<0.001
Serum pentosidine (ng/ml)*	33.8+12.8	19.4+6.6	<0.001
Prevalence of MONW**	65(65.0)	2(1.96)	<0.001

*****Mean ±SD, ** n(%), NS: p >0.05*

Table 2. Correlation analysis of pentosidine with metabolic risk factors

Variables	r	P-value	
Age in years		0.202	<0.01
Body mass index		0.293	<0.01
Waist circumference		0.328	<0.001
Hypertension		-0.209	<0.01
S. triglycerides		0.170	<0.01
S. HDL- cholesterol		-0.257	<0.01
Fasting S. glucose		0.234	<0.01
S. uric acid		0.194	<0.01
Fasting insulin		0.509	<0.001
HOMA-IR		0.557	<0.001

Table 3. Mean± SD of clinical and biochemical data of MONW and non- MONW subjects

Variable	MONW	Non- MONW	P. value
Age (year)	27.6 ±5.4	25.9 ±4.4	<0.01
Body mass index (kg/m^2)	24.4±2.1	23.4±2.1	< 0.05
Waist circumference	84.4±8.7	76.4±7.1	<0.01
(cm)	103.2 ±56.1	92.8 ± 48.4	<0.05
S. triglycerides (mg/dl)			
S. HDL- cholesterol (mg/dl)	37.9 ± 9.5	40.2 ± 10.3	<0.01
Fasting S. glucose (mg/dl)	107.8 ±40.6	88.4 ± 13.2	<0.001
S. uric acid (mg/dl)	4.2 ± 1.1	3.8 ± 0.9	< 0.01
Insulin (µlU/ml)	10.8 ± 2.6	5.9 ± 1.6	<0.001
HOMA-IR*	2.8 ± 1.1	1.2 ± 0.3	<0.001
S. Pentosidine (ng/ml)	40.0 ± 11.3	22.3 ± 5.3	<0.001

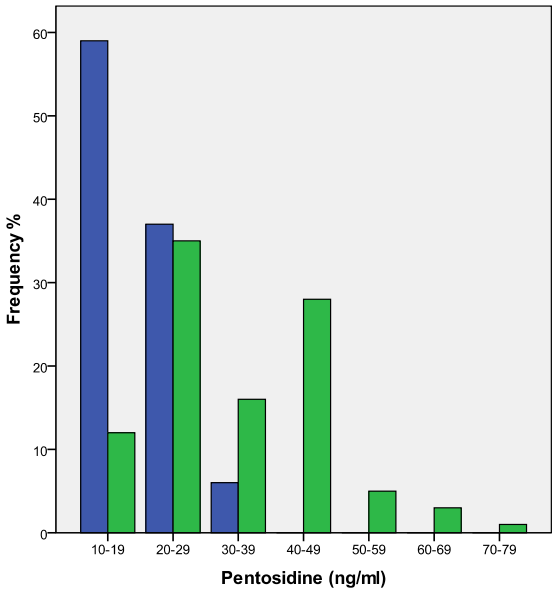

Figure 1. Distribution of serum protein pentosidine in positive and negative family history of type 2 diabetes (Positive, Negative).

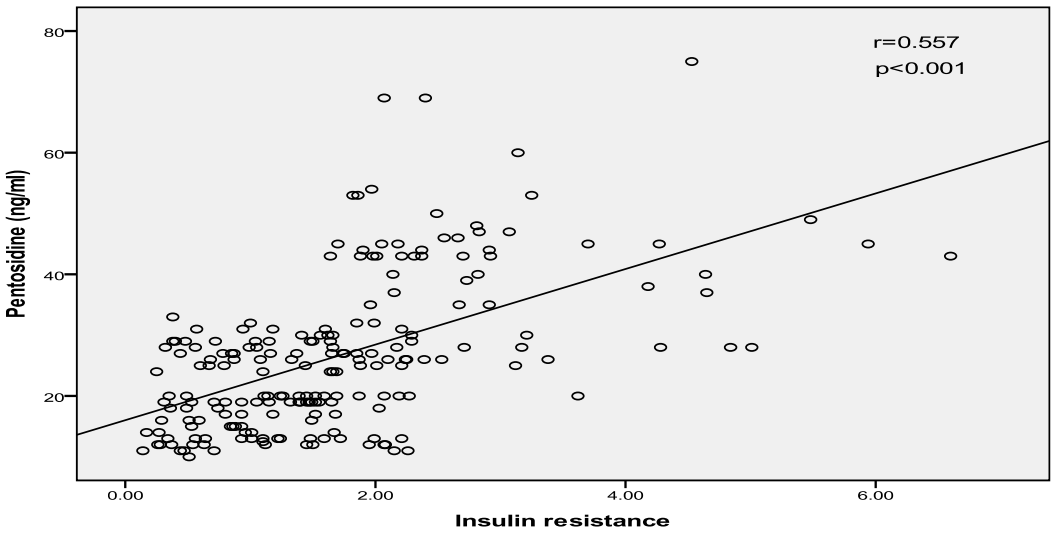

Figure 2. The correlation between serum pentosidine and insulin resistance

This finding represents an important extension of previous finding that ethnic groups with high propensity for diabetes are markedly hyperinsulinemic with fasting glucose within the normal range.10Indeed, fasting hyperinsulinemia known to reflect decreased insulin sensitivity and decreased insulin secretion together constitute the strongest independent predicator of type 2 diabetes.

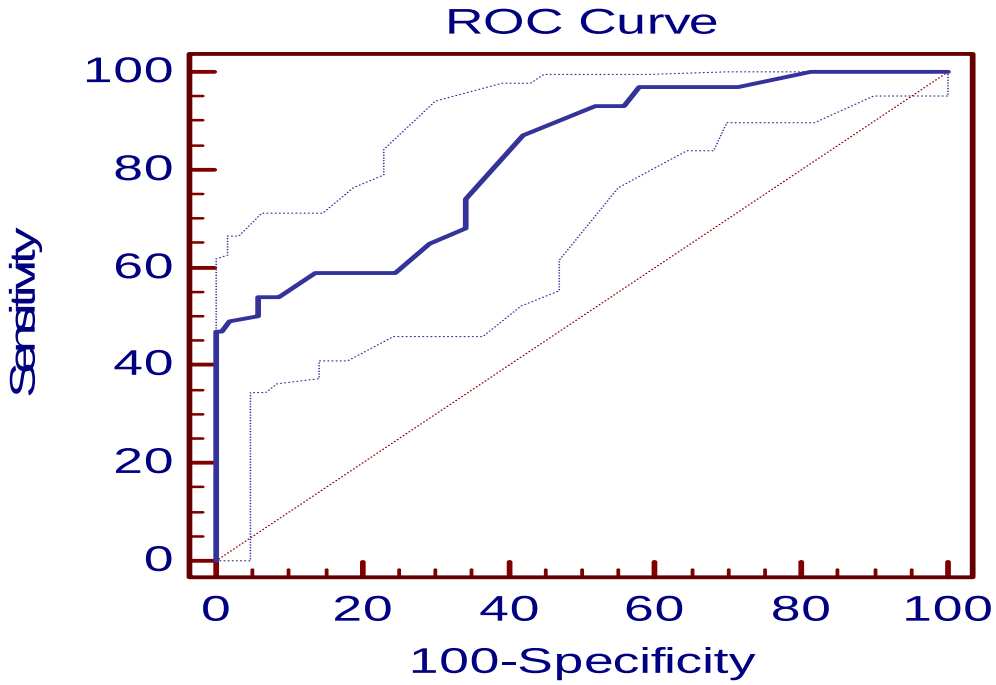

Figure 3. ROC Curve showing that trade- off between sensitivity (true positivity) and 100- specificity (true negativity) for serum protein pentosidine level (ng/ml) when used as a test for difference between positive family history of type 2 diabetes and negative family history.

The mechanisms for the increased pentosidine among the offspring of individuals with type 2 diabetes mellitus are not clear.[11] Glycated insulin has been identified in the pancreas of normal and diabetic animal models; the process of glycation in the beta cell is rapid and occurs in a time- and concentration-dependent manner.[12] Glycated insulin has a reduced ability to regulate plasma glucose homeostasis in vivo and to stimulate adipose tissue lipogenesis or glucose uptake and oxidation by isolated diaphragm and abdominal muscle in vitro.[13,14]

Studies in healthy human volunteers using the hyperinsulinaemic euglycaemic glucose clamp technique suggest that glycated insulin contribute to insulin resistance in type 2 diabetes mellitus.[15] The same process may occur in the body, and final result of this reaction is pentosidine. Pentosidine accumulates over time, and pentosidine formation and accumulation is greatly accelerated with high levels of circulating sugars and oxidative stress. It has been reported that

the increased concentrations of pentosidine found in diabetes are generally accepted to be a consequence of hyperglycemia.[16] However, still permissible to speculate that accumulation of pentosidine in various tissues may contribute to insulin resistance which associated with a number of clinical and metabolic abnormalities. Pentosidine is a highly sensitive marker for all advanced glycation end products. Serum levels of this so- called senescence cross link were found to be raised in patients with diabetes mellitus and more overtly in renal failure.[17] However, none of these reports directly investigated the level of serum protein pentosidine in first degree

relatives of type 2 diabetics and in MONW subjects.

The present study (a case- control study) reports on the levels of serum pentosidine of first- degree relatives of type 2 diabetics affected by MONW parameters. The results of this study showed that there were significant differences between MONW and non- MONW groups concerning the pentosidine. The most often approach for assessing the risk of diabetes, particularly in large population studies, is the high risk approach that usually includes first degree relatives of type 2 diabetic patients; Thus, this study was conducted on two identities, MONW and first degree relatives of type-2 diabetic patients, both are being under more attention in the scientific research as both are young, less likely to undergo medical intervention, more likely to benefit from medical interventions and when combined together, are less likely to be detected because of their apparently normal weight.

To determine the risk stratification for serum pentosidine in first- degree relatives of diabetics, ROC curve analysis was tested according to the trapezoidal rule.18 At a cut- off value of 29 ng/ml, a good specificity and positive predictive value was observed. This finding likely indicated that changes in serum protein pentosidine level are related to MONW parameters in offspring of individuals with type 2 diabetes mellitus. It must be noted that the present study has limitations. First, the study involved a small number of subjects and the results must be confirmed in a large sample. Insulin resistance has been measured indirectly only, although good correlation between HOMA insulin resistance and the values of serum protein pentosidine was obtained. Glycated hemoglobin (HbA1C) was not used as variable in the present study by reason of only 10 subjects were previously undiagnosed asymptomatic diabetes mellitus. Whatever was the reason or defect, the present study agrees with previous studies that off spring of type 2 diabetics are at increased level of AGEs. Further studies of large population including different level of relatives of type 2 diabetic patients should be investigated.

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پوختە

ئاستێ پروتینێ پێنتوسیدین لدەڤ نفشێ نەخوشێن شەکرێ ژجورێ دوێ

پێشەکی وئارمانج:بلندبوونا ریژا پروتینێ پێنتوسیدین دگەل نەخوشیا شەکرێ دیاربوویە کو ئێکە ژ ئەنجامێن بلندبوونا شەکرێ. نفشێ نەخوشێن شەکرێ بێن دمەترسیا بلندبوونا ریژا شەکرێ دا. ئارمانجا ڤەکولینێ پیڤانا ئاستێ پروتینێ پێنتوسیدین لدەف کەسێن ئێشا شەکرێ دخێزانێن وان دا هەی وەک پێنگاڤەک بو مەترسیا نەدروستیێن میتابولیکی لدەف وان.

ریکێن ڤەکولینێ: دووسەد ودوو کەسێن ساخلەم بەشداری ڤێ ڤەکولینا ژجورێ نەخوش و کونترول بوون. ژوان 100 نفشێ نەخوشێن شەکرێ بوون ژجورێ دوێ (50 کور و50 کچ) و یێن دی 50 نێر و 50 مێ کارمەندێن کولیژا پزیشکی بوون کو نەخوشیا شەکرێ دخێزانا وان دا نەبوون. پێزانینێ، کەساتی هاتنە وەرگرتن و نمونەکێ خوینی بو پیڤانا ئاستێ پێنتوسیدین، گلوکوز، رویـن و ترشێ یوریك هەر وەسا ئاستێ ئنسولینی.

ئەنجام: ئاستێ پێنتوسیدینی دخوینێ دا ببەرچاڤ یێ زێدە بوو لدەف کەسێن ئێشا شەکرێ ژجورێ دوێ دخێزانێن وان دا هەی. پەیوەندییەکا ئەرینی هەبوو دناڤبەرا ئاستێ پێنتوسیدین و فاکتەرێن مەترسی بو نەدروستیێن میتابولیك. پەیوەندییەکا بەرچاڤ هەبوو دناڤبەرا ئاستێ پێنتوسیدین و دژاتییا ئەنسولینی.

دەرئەنجام:وەك دەرئەنجام ئاستێ خوینێ یێ پێنتوسیدین رێکەکا باشە بو لێگەریانێ لدور فاکتەرێن مەترسیێ بو نەدروستیێن میتابولیك لدەف نفشێ نەخوشێن شەکرێ ژجورێ دوێ.

الخلاصة

مستويات بروتين البينتوسيدين لدى نسل مرضى السكري من النمط الثاني

الخلفية والأهداف: ان ارتفاع مستوى البينتوسيدين لدى مرضى السكري مرتبط بزيادة نسبة السكر في الدم و نسل مرضى السكري معرضين لخطورة ارتفاع نسبة السكر لديهم. الهدف من البحث هو قياس نسبة البينتوسيدين لدى الأشخاص الذين لديهم تاريخ عائلي لمرض السكري كخطوة لتقييم خطورتهم للامراض الميتابوليكية

طرق البحث: تم اشراك 202 شخص سليم للبحث من نمط الحالة والشاهد. 100 منهم كانوا ممن لديهم تاريخ عائلي لمرض السكري و 102 كانوا من كادر كلية الطب ممن لا يوجد مرض السكري في عوائلهم. اضافة للمعلومات الشخصية تم أخذ نموذج الدم لقياس نسبة البينتوسيدين، الكلوكوز، الدهون وحامض اليوريك، اضافة الى قياس مستوى الانسولين.

النتائج: وجدت زيادة ملحوظة في مستوى البينتوسيدين لدى من لديهم تاريخ عائلي للسكري من النمط الثاني مقارنة بعينة الشاهد. كما وجدت علاقة ايجابية بين مستوى البينتوسيدين وعوامل الخطورة الميتابوليكية، و كانت هناك علاقة معنوية قوية بين مستوى البينتوسيدين و مقاومة الانسولين.

الاستنتاج: يمكن استخدام مستوى البينتوسيدين كطريقة جيدة للكشف عن عوامل الخطورة الميتابوليكية لدى نسل مرضى السكري من النمط الثاني.

THE EFFECT OF LEVONORGESTREL INTRAUTERINE SYSTEM IN THE MANAGEMENT OF HEAVY MENSTRUAL AND IRREGULAR VAGINAL BLEEDING

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ABSTRACT

Objective: To assess the effect of a Levonorgestrel releasing intrauterine system in the management of heavy and irregular menstrual bleeding.
Design: A prospective study.
Setting: The cases were collected from a private clinic in Dohuk city/ Kurdistan Region.
Methods: Sixty patients with a failed trial of medical therapy and awaiting hysterectomy were treated with a Levonorgestrel Intrauterine System (LNG-IUS). The menstrual loss was estimated by the number of sanitary protection devices used and the degree of staining. Further assessment was made by any concomitant menstrual clots, flooding and the associated dysmenorrhoea that affecting their life or job. The follow- up was carried out at 3 months (first visit), 6 to 9 months (second visit) and up to 5 years period.
Results: Out of the 60 patients, 45 were pleased with the results of using the LNG-IUS. There was no expulsion at the time of insertion. During the follow-up, LNG-IUS was prematurely removed from 8 patients before the 1st visit due to different side effects (pain, backache, continuous vaginal bleeding) and 5 patients did not attend at all. Around the end of the first year, 2 patients had their removal because of bleeding problems. Most of the patients had unscheduled bleeding for six to eight weeks post-insertion. Amenorrhoea was seen in 10 patients and the improvement of the premenstrual syndrome recorded in 70%, with a satisfaction rate of 75%. The reduction in dysmenorrhoea was in 85% of the patients.
Conclusion: The LNG-IUS is an effective nonsurgical treatment for the management of heavy and irregular menstrual bleeding and dysmenorrhoea, with high rates of continuation and satisfaction, low rates of side-effects and complications that has an additional benefit as a contraceptive and in relieving of premenstrual syndrome.

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Key words: Levonorgestrel, heavy menstrual, vaginal bleeding

Heavy menstrual bleeding (HMB) is defined as excessive blood loss (over several consecutive cycles).It is now the preferred description for abnormalities of blood loss, due to some confusion over the various terminology used for abnormalities of menstrual blood loss. It has replaced the older term 'Menorrhagia'. HMB is a major clinical problem, with significant effects on the quality of women's life .[1,2,3] The objective definition of HMB is a blood loss of greater than 80 ml per menstruation, but it is usually women's perception of their own bleeding which dictates referral and subsequent treatment. [4,5]

HMB affects one in three women of reproductive age .[5,6] In the UK, around 1.5 million women per year consult their general practitioner with menstrual complaints.[6] It accounts for around 12% of all referrals to gynecology outpatients in Australia and other Western countries. [7]

The causes of HMB are: fibroids, polyps , coagulopathy, malignancy, thyroid disease, pelvic infection, arteriovenous malformations, iatrogenic (drugs, copper intra-uterine devices) and bleeding of endometrial origin, which has previously been termed as 'Dysfunctional Uterine Bleeding' (DUB).[8]

Various treatments for HMB with no organic causes (endometrial origin) have been used. Medical therapy in form of (Antifibrinolytics, Prostaglandin synthetase inhibitors, Combined oral

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contraceptive pills and Norethisterone) has limited effectiveness, due to poor patient compliance, adverse side effects and the cost of medications. [5-8]
Hysteroscopic endometrial ablation has proved to be an attractive option but can be technically difficult and may result in serious complications .[8,9] Hysterectomy is associated with significant morbidity expense, prolonged hospital stay and recovery time .[9]
One of the treatment option emerged in the last years for the management of HMB is a Levonorgestrel-Releasing Intrauterine System Mirena®, (LNG-IUS). The Mirena has a capsule containing Levonorgestrel 52mg around its stem which releases a daily dose of 20 micrograms of hormone. Used originally as a contraceptive method. It causes atrophy of the endometrial glands, decidualisation of the stroma, thickening of the cervical mucus and desensitization of the endometrium to estrogen, so it is an excellent alternative to surgery for a women with HMB who also seek reliable long-acting reversible contraception with an effective lifetime of 5 year .[5,10]
The use of LNG-IUS is associated with little or no vaginal bleeding.
Randomized controlled trials show that Mirena will reduce menstrual loss by up to 96% after 1 year but that full benefit may not be seen for 6 months of insertion. [11,12] Another study revealed a reduction in menstrual blood loss by up to 90% with 85% satisfaction,[13] there is a significant reduction of clotting,flooding,less anaemia and improve the quality of life .[14]
As its action is local, progestogene - related side effects(abdominal pain, mastalgia, weight gain , backache, headache, anxiety) are much less than with oral agents .[15]Women should be fully counseled that they are likely to experience unscheduled spotting/bleeding during the initial months of use .[16,17]In UK study ,10.5% of new cases of LNG-IUS ceased use by the end of the first year owing to bleeding problems. [18] Primary and secondary dysmenorrhoea are greatly reduced with using this system, in addition to its effect for the improvement of the premenstrual syndrome. [19]
The LNG-IUS may be inserted in the outpatient setting and requires change every 5 years ,the expulsion rate is low (2-5%) but is more likely to occur in the few weeks after fitment. [5]
The LNG-IUS is licensed for contraception, treatment of HMB , DUB, dysmenorrhoea and a part of hormone replacement therapy (HRT) regime in conjunction with systemic estrogen, by providing endometrial protection.[20]

METHODS

Ethical approval for the study was obtained from the Human Research Ethics Committee a Directorate General of Health –Duhok Governorates. The consent form had applied for each patient in the study.

Sixty eight patients were recruited into the study over a period from January 2004 till January 2009, at private clinic in Duhok city/ Kurdistan region of Iraq. The study concentrated on 60 patients of them with menstrual disorders, the other 8 patients were excluded as the insertion was purely for contraception reasons.

Most of the patients had tried one or more forms of medical treatments which are used for this problem. The majority received a combination of prostaglandin synthetase inhibitors and antifibrinolytic drugs.

The age range was 28 to 53 years (mean 39.8years; median 40 years); 58 of the patients were parous and 2 nulliparous; 9 patients had been sterilized; 44 were using barrier with natural methods and 7 were not sexually active.

Assessment of the menstrual blood loss was estimated by the number of sanitary protections used, the degree of staining, concomitant menstrual clots, flooding, soiling of the under-wears and cloths

which might affect the normal life. All patients were asked about associated symptoms like dysmenorrhoea and deep dyspareunia. Vaginal ultra-sound scanning was performed for all patients to assess the endometrial thickness and to exclude any obvious organic causes. Endometrial sampling was done to exclude premalignant or malignant changes and a note was made about the length of the uterine cavity.

The LNG-IUS (Mirena; Schering, Bayer, Finland), was inserted aseptically at the end of a menstrual period. The degree of difficult was recorded as either being easy, moderate or difficult and whether local anesthesia was necessary for insertion of the device. If required, 10 ml of local anesthetic gel was inserted into the cervical canal for minutes before insertion of the LNG-IUS. [14]

Each patient was reviewed at three months(first visit), between six and nine months (second visit) , then follow- up until 5 years. Each patient was asked to use the same sanitary protection throughout the study to standardize the observed loss. The percentages were calculated according to the intention-to-treat model.

RESULTS

In most parous women (n =54) LNG-IUS insertions were classified as easy, with only 2 recorded as moderately difficult and 2 as difficult. In nulliparous women 1 insertion was considered easy and 1 moderately difficult. Local anesthesia was necessary in 2 parous patients. There was no expulsion of the device during insertion.

Of the 60 patients, 48 (80%) had the device inserted for regular heavy periods, 11 (18.3%) for irregular heavy periods and 1 (1.7%) for inter-menstrual bleeding. Forty-two (70%) of the 60 patients had tried other medical therapies for menstrual dysfunction before the insertion of LNG-IUS in the following categories: 29 (48%) having tried one form of medical therapy,12 (20%) having tried two forms and 1 (2%) having tried three types. Eighteen (30%) had the LNG-IUS inserted as first-line management. (Table 1) shows the different types of medication used prior to loop insertion.

Table 1 . Previous medical management of menstrual dysfunction

Medical treatment	Number of women (%)
Progestogens	**25 (36)**
Tranexamic acid	**15 (22)**
Non-steroidal anti-inflammatory drugs	**19 (27)**
Combined oral contraceptive	**10 (14)**
Hormone replacement therapy	**1 (1)**
Total	**70**

Thirteen (13) women were withdrawn from the study just before the first visit: five (5) did not attend the follow up; the device was removed in eight (8): three (3) because of severe lower abdominal pain and backache, two (2) had spontaneous expulsion of the device during their heavy cycle and both asked to be put on the waiting list for surgery, three (3) found the unscheduled loss unacceptable and requested the removal of the device; (although most of the patients experienced some unscheduled bleeding and requiring

the use of sanitary protection during the first six to eight weeks post-insertion),one ended with hysterectomy and GnRH analogues were given for the 2 others(as this kind of medication was not tried before). After a mean period of 10.5months (range 9–12 months) two patients continued on
having the vaginal bleeding, there was no respond to the device for which removal was mandatory and they ended with hysterectomy. For the sake of pregnancy, 2 patients requested the removal of the system after mean period of 18 months. (Table 2).The remaining 43 (71.7%) women continued their follow up regularly and were pleased with the results. The mean duration of use was 51 months (range 42 to 60 months).During the follow up: 10 (16.7%) became amenorrhoeic, 26

Table 2 . Reasons for discontinuation of LNG-IUS

Case	Insertion-removal Interval	Reasons for dissatisfaction	Treatment
Before the 1st visit:			
1	**2 months**	**Pain, backache.**	**Nil**
2	**2 months**	**Pain, backache.**	**Nil**
3	**2 months**	**Pain, backache.**	**Nil**
4	**2 months**	**Heavy bleeding (expulsion)**	**Hysterectomy**
5	**2 months**	**Heavy bleeding (expulsion)**	**Hysterectomy**
6	**2 months**	**Spotting continuous bleeding**	**GnRH analogues**
7	**2 months**	**Spotting continuous bleeding**	**GnRH analogues**
8	**2 months**	**Spotting continuous bleeding**	**Hysterectomy**
After the 2nd visit:			
9	**9 months**	**Continuous bleeding**	**Hysterectomy**
10	**12 months**	**Irregular heavy spotting**	**Hysterectomy**
11	**16 months**	**Wanted pregnancy**	**Nil**
12	**18 months**	**Wanted Pregnancy**	**Nil**

(43.3%) described occasional bleeding and 7(11.7%) had regular cyclical bleeding. It was apparent that there was a considerable reduction in menstrual blood loss (oligomenorrhoea).
The removal rate as a result of bleeding causes was 7 (11.7%) and 5(8.3%) for other causes. Important observation was that 85% of patients had a reduction in the associated dysmenorrhoea, With 70% improvement in the premenstrual syndrome.

Regarding the adverse effects experienced by the patients; 22 (47%) reported various adverse effects; some had more than one as shown in (Table 3).
Weight gain was the most common reported symptom 16 (34%) of the 60 women: 45 (75%) women felt they would like to use a LNG-IUS again, 10 (17%) would not (including those who had it removed) and 5 (8%) were unsure. (Table 4)

Table 3 .Adverse effects experienced while Levonorgestrel releasing Intrauterine System in situ after the 1st visit

Adverse effects	Number of women		
	Increased	Decreased	Unchanged or nil
Abdominal pain	8	19	20
Mastalgia	3	17	27
Headaches	6	15	26
Weight increase (1–5 kg)	10	0	0
Weight increase (6–10 kg)	6	0	0
Weight increase (>10 kg)	0	0	31
Anxiety	5	7	35
Backache	4	8	35
Nausea	4	0	43

Table 4. Satisfaction rates with levonorgestrel releasing intrauterine system

	Number of women (%)
Satisfied	45 (75)
Dissatisfied	10 (17)
Uncertain	5 (8)

DISCUSSION

In the current study, there was no expulsion noticed for the device spontaneously at time of insertion. This finding was in contradictory to the results reported by Crosignani PG et al. and Barrington JW with a high rate of 4-8% and 12% of expulsion respectively, [12,13] and to 1% in another study in Newzealand. [7] Our finding of 3.3% expulsion rate at the second month of insertion was similar to 2-5% rate in UK which occurred in the few weeks after fitment. [5]

Regarding the use of medical therapies, 70% of the patients had tried other medical therapies for menstrual dysfunction before the insertion of LNG-IUN, and 30% had the device inserted as first line of management, this result was contrastive to the study had done in Newzealand, where 49% of patients had tried medication prior to insertion and 51% had the device inserted as first line of management. [7] Another study in UK, 48% had a direct insertion for the device as a first mode of management for DUB, with medical therapies in 52% of the patients. [21]

Excessive and/or continuous vaginal bleeding was the most common indication for removal of the device , our finding 11.7% was in agreement with the results of Finnish study [16], but was contrasting to the finding had seen in Palmerston North Hospital in Newzealand ,with a low rate of 7% removal of the loop as a cause of continuous bleeding , [7] and to a high rate of 16.7% in UK .[8,18]

It was thought that adverse hormonal side-effects were experienced most commonly in the first year after insertion, but there were reports of higher rates of them with prolonged use of the LNG-IUS. However, they rarely led to premature removal of the device .[16,17] The total incidence of side-effects that has been described by

Lahteenmaki P et al, was 56%.[18] In Palmerston North Hospital, 36 patients out of 69(52%) reported that they had experienced adverse effects that might be considered hormonal, one patient cited a possible hormonal side-effect (migraine) as an indication for removal of the system. [7] These were not corresponding to the current study in which , 47% was the total incidence of side effects. Weight gain was the most common one noticed by users as 34%.

Amenorrhoea was noticed in 16.7% of women, Oligomenorrhoea in 55% (either in form of regular cycles with less blood or occasional bleeding).This finding disagree with 65% oligomenorrhoea rate in a study by Crosignani PG et al, [13] and rates of amenorrhea of 8% and 12% in others studies had been previously published by Barrington J W and Da Silva MO. [13,15] the same difference with another study that 64% oligomenorrhoea and 29% amenorrhea which had been published later in Australia and Newzealand .[7]

This study had demonstrated a continuation rate for LNG-IUS of 71.7%, which disagreed with 50% found by Rani Nagrani C. et al. [22]About the satisfaction rate, 75% of our patients had reported satisfactory respond to the device ,these finding where in congruent with the findings reported by Palmerston North Hospital 76% [7] and Crosignani PG et al, 72%.[13]A high rate of satisfaction had reported by Lahteenmaki P et al, 80%.[15]

The average age of women in this study was 40 years and therefore the device would only need to be changed once or twice at the most before the end of naturally occurring period.

CONCLUSION

In conclusion, the LNG-IUS offers a simple and effective alternative to surgical treatment for abnormal and heavy vaginal bleeding with a concomitant reduction in surgical morbidity and mortality. In addition to its benefit in relieving dysmenorrhoea and premenstrual symptoms for women in our region. With high rates of continuation, satisfaction and low rates of side-effects and complications.

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پوختە

خواندنەك لدور پێچاوپێچێ هۆرمۆنی (مارینا) د چارەسەرکرنا خوین بەربوونا پیساریێ وتێکچوونێن خوینا هەیڤانە.

ئەڤ خواندنە هاتەکرن بو زانینا کارتێکرنا پێچاوپێچێ هۆرمۆنی (مارینا) د چارەسەرکرنا زێدەبوونا تێکچوونێن خوینا هەیڤانە، پیسار ونەخۆشیێن وێ، ئەڤە ل کلینیکەکا تایبەت ل باژێرێ دهوکێ ل هەرێما کوردستانێ هاتەکرن.

ڕێکا خواندنێ: 60 ئافرەت کەتنە بەر خواندنێ کو خوین بەربوونا پیساریێ و تێکچوون ونەخۆشیێن وێ لنك هەبوون. پشتی چارەسەرکرنا وان بکار ئینانا دەرمانێن نوژدارێن یێن تایبەتمەند بو ڤێ چەندێ، ژڤان بو هاتنە دان ژبو نشتەرگەریا راکرنا مال بچویکی، چنکو شوینا وێ چ ڕێکێن دی نەبوون. بڕا خوین بەربوونێ ب ڕێکا دەستمالێن ساخلەمیێ یێن تایبەت بو ڤێ چەندێ هاتە پیڤان. زێدەباری تەڕبوون وبڕا مەیینا خوینێ وڕێژا پیسبوونا جلکێن دی وئەرێ چالاکی کاری ل چالاکییێن دی دکەت. زێدەباری زانینا دژوار یا ئێشێ یان ئەوا دبێژنێ نەخۆشیا پیساریێ. پشکنینا هیموگلوبینی وڕێژا ئاسنی هاتەکرن بەری و پشتی بکارئینانا پێچاوپێچی ئەوژی د سەرەدانا ئێکێدا پشتی (3) هەیڤا، پاشی سەرەدانا دووێ دناڤبەرا 6 – 9 هەیڤا.

ئەنجام: تێبینی هاتە کرن کو پێچاوپێچ یێ باشە وەك هۆکارەکێ باش بو چارەسەرکرنا خوین بەربوونا مال بچویکی لنك 40 ئافرەتا ژ 47 ئافرەتێن کەفتینە بەر هەلسەنگاندنا ئێکێ و پاش (5) ئافرەتێن دی یێن نەخۆش د هەلسەنگاندنا دووێدا. ژ ئەوێن ل هیڤیا ژڤانێ خو بو راکرنا مال بچویکێ (13) ئافرەتێن نەخۆش، هەلسەنگاندنێ ژ (5) ژ وان نەگرت و دوو ئافرەت ژی پێچاوپێچێ وان بخو هاتە دەرێ ژبەر زۆریا خوین بەربوونێ و (5) ژ وان پێچاوپێچ راکر ژبەر بەردەوامیا خوین بەربوونا پێچ پیچە وئێشێن پشتێ، پشتی دوو هەیڤا ژ دانانا وێ. پێچاوپێچ هاتە راکرن ژبەر بەردەوامیا خوین بەربوونێ نێزیکی سالەکێ لنك دوو ئافرەتێن نەخۆش، پیسار ب ئێکجاری نەما لنك (7) ئافرەتێن نەخۆش د ژیێ (50) سالیدا، دیاربوو کو ڕێژا وان ئافرەتا ئەوێن نەخۆشیا پیساریێ لنك پاش چێبووی گەهشتە 70 ٪ وڕێژا ڤی هۆکاری وەك چارەسەریەکا باش بو چارەسەرکرنا خوین بەربوونێ وتێکچوونێن خوینا هەیڤانە گەهشتە 75 ٪ بەلێ ڕێژا کاریگەریا هۆکاری ونەبوونا کێم وعەداڤێ 85 ٪ بوو.

دەرئەنجام: پێچاوپێچێ هۆرمۆنی هۆکارەکێ باش وکارتێکەرە وهەوجەی نشتەرگەریێ نینە بو چارەسەرکرنا خوین بەربوونا مال بچویکی و تێکچوونێن خوینا هەیڤانە زێدەباری کێمکرنا ئێشێن وێ وبکارئینانا وێ وەك هۆکارەك بو نەبحالبوونێ.

الخلاصة

دراسة حول دور اللولب الهورموني (المارينا) في علاج نزف الطمث واضطرابات الدورة الشهرية

هذه الدراسة اجريت لمعرفة تاثير اللولب الهورموني (المارينا) في علاج زيادة واضطرابات الدورة الشهرية(الطمث) وعسره . وقد تم اجراؤها في عيادة خاصة في مدينة دهوك من اقليم كردستان .

طريقةالدراسة: 60 مريضة ادخلت الدراسة تشكو من نزف واضطراب الطمث وعسره،

بعد فشل علاجهم باستعمال الأدويةوالعقاقير الطبية المخصصة لهذا الغرض و كان خيار عملية رفع الرحم هو الحل الأمثل لهم، لعدم توفر طرق ثانية بديلة.وقد تم تقدير كميةالنزف عن طريق احتساب المناديل الصحية الخاصة بهذا الغرض ونسبة ابتلالها وكمية الخثرالدموية الموجودة ونسبة اتساخ الملابس الاخرى وهل تؤثرعلى استمرارالفعاليات اليوميةوادائها. بالاضافة الى معرفة شدة الآلام المصاحبة له اومابعسر الطمث .وتمت متابعة المرضى بعد ثلاثة اشهر من وضع المارينا ثم 6–9 شهر ومن ثم بصورة دورية حتى نهاية السنة الخامسة.

النتائج: من 60 مريضة اللواتي ادخلن الدراسة، 45 مريضة استمرن في استعمال الوسيلة وقد ابدوا ارتياحهم منه.تم رفع اللولب في 8 مريضة قبل الزيارة الاولى نظرا لوجود عدد من المضاعفات مثل آلآم الظهر والنزف المستمر،مع وجود 5 مريضات لم يحضرن المتابعة بتاتا .في نهاية السنة الاولى تم رفع اللولب لدى 2 مريضة اخرى لكثرة النزف وعدم انقطاعه.ونتيجة لرغبة الحمل في 2 اخريات تم رفعه لديهم بعد مرور حوالي 18 شهرا. وقد انقطع الطمث بصورة نهائية عند 10 مريضات في العقد الخامس من العمر.وتبين ان نسبة اللوات لاحظن تحسن كبير في عسرالطمث هي85٪،ونسبة تقبل هذه الوسيلة كعلاج جيد لمعالجة نزف وإضطرابات الدورة الشهرية واستمرار اتباعها 75٪.مع ارتياح من اعراض ما قبل الدورة الشهرية وقلقها في 70٪.

الاستنتاج: اللولب الهورموني وسيلة جيدة ومؤثرة ولاتحتاج الى تداخل جراحي في معالجة الكثير من حالات النزف الرحمي واضطرابات الدورة الشهرية بالاضافة الى التقليل من آلآمها.كما يستعمل كوسيلة جيدة لمنع الحمل ايضا.

IMMUNOPHENOTYPIC ANALYSIS OF BONE MARROW DERIVED MESENCHYMAL STEM CELLS IN ALBINO RATS

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ABSTRACT

Background and Objectives Mesenchymal stem cells (MSCs) are pluripotent adult stem cells residing within the bone marrow (BM) capable of self renewal, production of large number of differentiated progeny and regeneration of tissue. This study aimed to isolating and culturing of the BM-MSCs from albino rats and detecting of the phenotypic nature of rat MSCs.
Methods The BM was collected from young male white rats and separated by gradient centrifugation, then the mononuclear cells (MNCs) were retrieved from the buffy layer and cultured in Modified Eagle,s Medium (MEM) supplemented with 10% Fetal calf serum (FCS) and incubated at 37Cº and 5% CO2. Then immunophenotypic analysis of MSCs were detected by using CD markers (CD 11b, CD34, CD71 and CD90).
Results After 10-12 days of primary culture, the MNCs derived MSCs was duplicating rapidly and showed fibroblast like morphology appearance. By the end of the second week the adherent cells formed monolayer which expanded by two passages. The results of immunophenotypic analysis of MSCs indicated that most of them express positive response for CD71, CD90 and negative response for CD11b, CD34.
Conclusion: The results of the present study indicate the major population of adherent cells are MSCs and these cells are positive for CD71, CD90 the MSCs specific markers, and negative for heamtopoeitic specific markers (CD11b, CD34) .

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Key words: Bone marrow stromal cells, Proliferation, Immunocytochemistry analysis, *In vitro*.

Stem cells have been regarded as undifferentiated cells capable of proliferation, self-renewal, and production of a large number of differentiated progeny [1]. Bone marrow (BM) can be collected from adults and used for transplantation without posing ethical questions or creating problems of tissue matching and rejection [2]. BM transplantation is normal operation which is used for treatment of many diseases [3]. There are at least two populations of adult stem cells that have been identified in the BM which represented by hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). MSCs are pluripotent adult stem cells residing within the BM microenvironment, it has an adherent nature and expandable in culture and can be differentiated into osteoblasts, chondrocytes, neurons, skeletale muscle cells and cardiomyocytes [4].

Identification has relied on morphological and molecular indications of functions, such as expression of specific enzymes. The surface of every cell in the body are coating by specialized proteins called receptors that have the capability of selectively binding or adhering to other signaling molecules [5] . Bone marrow – derived stem cells include both HSCs and MSCs. Unlike HSCs, the MSCs are CD34- and CD45-. Other cell – surface markers characteristic of MSCs include CD29, CD44, CD90, CD106, CD120a, CD124, SH2, SH3 and SH4 [6,7]. The development of series of monoclonal antibodies raised towards surface mesenchymal progenitor cells (MPCs) antigens, along with other antibodies developed to characterize BM stem cells has been crucial for the immunophenotyping of these cells.

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Mesenchymal progenitor cells do not express the typical hematopoietic antigens CD45, CD34 and CD14 [8]. So, the present study aimed at isolating and culturing of the BM-MSCs from albino rats Rattus rattus norvegicus albinos and detecting of the phenotypic nature of rat MSCs by using CD 11b, CD34, CD71 and CD90.

METHODS

Cell isolation and culturing

Young male rats (Rattus rattus norvegicus albinos) age (50-55) days were used as an animal model for the isolation of MSCs from the BM. These animals were obtained from the animal house in Medical Research Center / College of Medicine / Al-Nahrain University / Baghdad.

Bone marrow was extruded from femur and tibiae by using a syringe with 20-gauge needle and mixed with 3mL culture medium (Modified Eagle,s Medium) (MEM) supplement with 10% Fetal Calf Serum (FCS). Then centrifuged at 2000 rpm for 10 minutes. After centrifugation, the fat and serum layers were discarded and the cell pellet was resuspended with 3ml of culture medium.

To separate BMCs and red blood cells, the gradient centrifugation method described by Yablonka-Reuveni and Nameroff, (1987) was used [8]. The cell suspension was loaded carefully onto 5ml of 60% Percoll in sterile conical tube, and then was centrifuged in a cooling centrifuge for 20-25 minutes at 2000 rpm at 8Cº. After density gradient centrifugation the mononuclear cells (MNCs) were retrieved from the Buffy coat layer by sterile Pasteur pipette. Then washed two to three times with phosphate buffer saline (PBS) by centrifugation at 2000 rpm for 10 minutes at 8Cº.

The cell suspension of MNCs were seeded in 50 cm2 culture flasks with 5 mL of culture medium supplement with 10%FCS at a plating density of 1x106 cells/mL. The culture was maintained in an environment of 37Cº and humidified atmosphere with 5% CO2 for two weeks, with 50% of the media being changed every 3 days. Cultures were screened continuously to get hold of the cell development and growth.

After 10 days, the primary culture of MSCs reached nearly 70-80% confluence. Culture medium was aspirated and the cells were detached by incubation with 2ml of 0.25% trypsin-EDTA for 5-10 minutes at 37Cº. The cell suspension was centrifuged at 2000 rpm for 10 minutes then the supernatant (medium) was aspirated and the cells pellet was resuspended in 1ml of culture medium. The cell suspension was plated as ratio 1:2 in plastic tissue culture flasks. The cultures should be passage when the MSCs have reached an approximately 80% confluence [10].

Immunophenotyic analysis of mesenchymal stem cells:

The second passage of rat BM-MSCs were resuspended after trypsin treatment and seeded into 4-well culture plates at density of 1x104 cell /well. After the cells had grown to near confluences, the attached cells were washed three times with PBS and fixed with 4% Phosphate buffered formalin for 10 minutes, then detected by immunocytochemistry method, which was performed with mouse monoclonal antibodies against human CD 71 and CD 90 for detection of MSCs and CD 11b and CD 34 for detection of HSCs according to the method of (`10, 11).

RESULTS

Morphology of mesenchymal stem cells in primary culture

After three days of primary culture, the MSCs were attached to the culture flask sparsely and this cells displayed a spindle – like shape (Figure 1A). At seven days cultivation numerous fibroblast like- cells were observed. These cells gradually grow to form small individual colonies

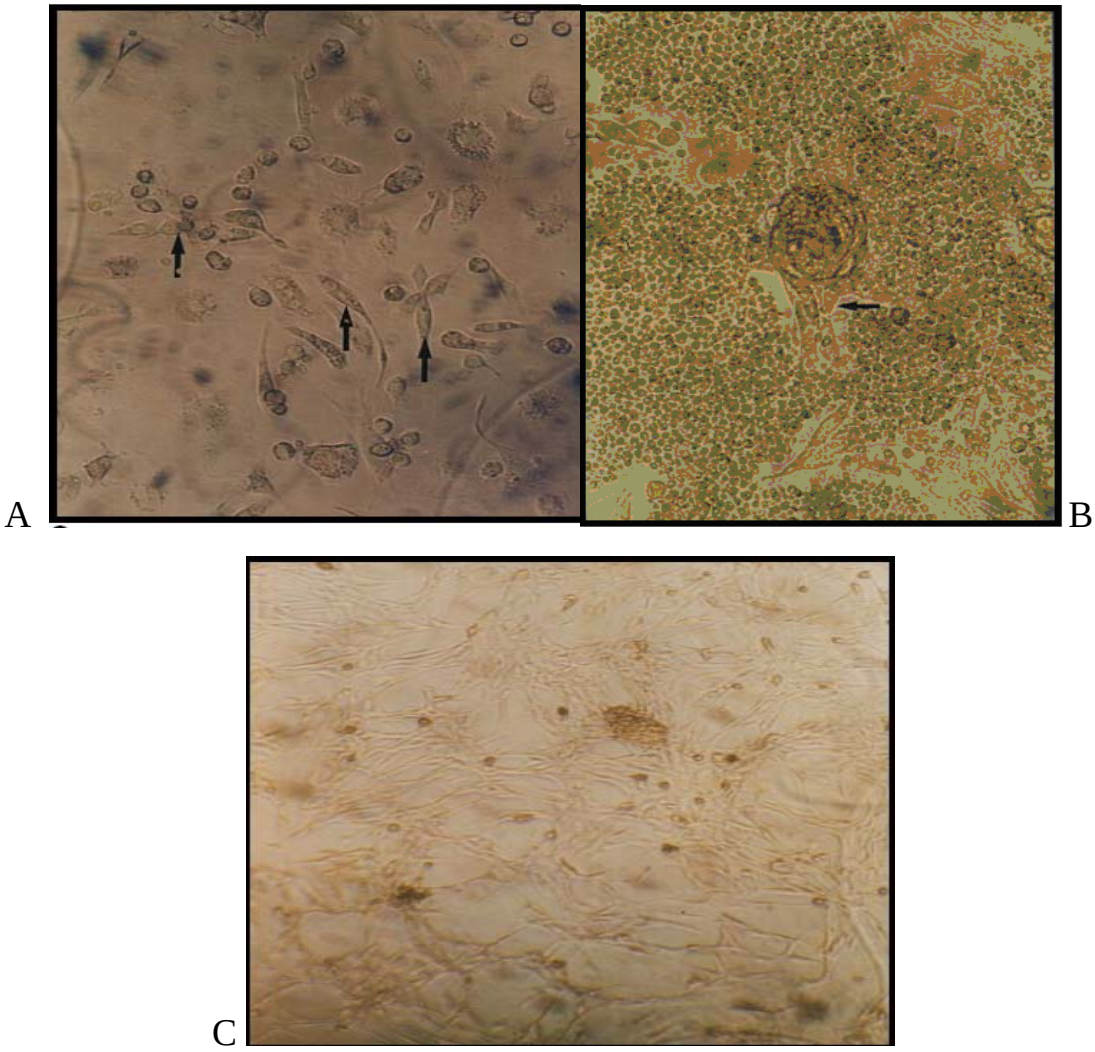

Figure 1. The morphology of BM-MSCs, (A): The cells after three days of culture, MSCs began to proliferate (arrow) (X160). (B):The cells at seven days of culture showed the formation of the large colony of MSCs surrounded by spindle like cells (arrow) (X160). (C): The cells in the end of second week of culture showed that the cells more expanded and formed a monolayer of adherent cells (X100.8).

displaying fibroblast – like morphology with short and long processes (Figure 1B). By day 10, the MSCs was duplicating rapidly and the cells morphology was mainly spindle shaped. By the end of the second week, the adherent cells reached nearly 70-80% confluence and formed monolayer of adherent cells and this layer was expanded by two passages (Figure1C).

Expansion of mesenchymal stem cells:

The passaged MSCs behaved similarly to those in primary cultures, however, the cells are larger in size and more homogenous in morphology. Grossly, the MSCs in subcultures could be divided into two types of cells small spindle or triangle-like cells and broad flattened cells (Figure 2A).When the first passage become nearly confluence, the cells recultured in similar conditions for second passage (Figure 2B).

Immunophenotype analysis of mesenchymal stem cells

To determine the phenotypic nature of rat MSCs, the surface antigen CD11b, CD34, CD71, and CD90 were examined by immunocytochemistry staining technique.

The results of immunophenotypic analysis showed that more than 90% of the BM-MSCs were strongly stained with CD71 (Figure 3A) and CD90 (Figure 3B). The stained cells with brown granular DAB reaction product in the cytoplasm were considered positive for both surface

antigen CD71+ and CD90+ . Besides, these cells represented the undifferentiated state of rat MSCs.
In contrast, a majority of adherent cells are color of counter stain Harris hematoxylin.

negative for CD11b- (Figure 3C) and CD34- (Figure 3D) and stained with blue
Thus we confirmed that the majority population of adherent cells are MSCs.

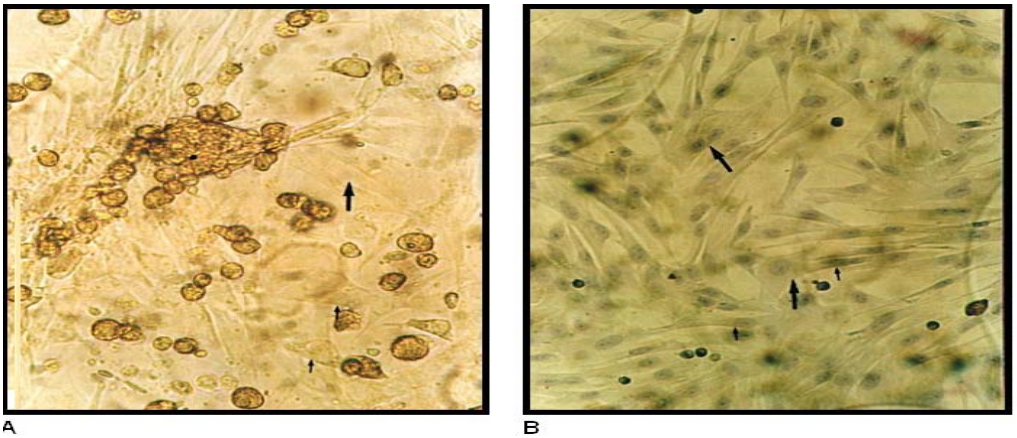

Figure 2. Expansion of BM-MSCs. (A): Two days after first passage, this figure showed the appearance of two types of cells in culture: spindle-like cells (thick arrow) and broad flattened cells (thin arrows) (X160). (B): The cells after first week from the first passage, the monolayer become nearly confluence (X160).

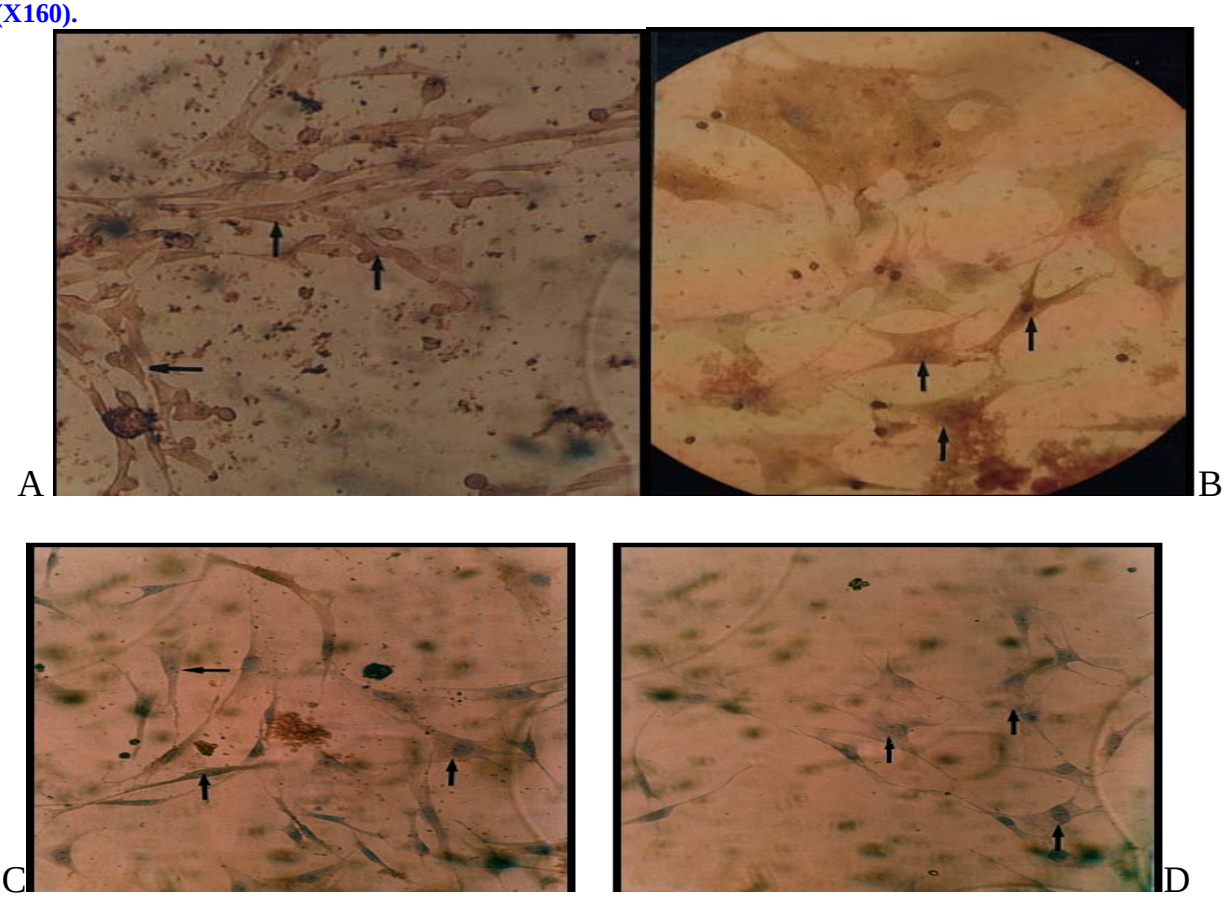

Figure 3. Immunophenotypic analysis of rat MSCs on the second day of the second passage. (A): the most of adherent cells MSCs were positive response for CD71 marker and stained with brown color DAB stain (arrows) (X100.8). (B): the MSCs were positive response for CD90 marker and stained with brown color (arrows) (X160). (C): the cells MSCs were negative response for CD11b marker and this cell stained with blue color of counter stain haematoxylin (arrows) (X100.8). (D): the cells of MSCs appeared negative response for CD34 marker and stained with blue color (arrows) (X100.8).

DISCUSSION

The most widely used separation liquid for isolation cells on density gradients has been Ficoll but this separated liquid has a number of disadvantages which are presented their low stability on storage, their coast and difficulties in the preparation of gradients [13], so in the present study, Percoll was used to replace the separated liquid. Percoll is a commercially available material for density gradient centrifugation of cells and subcellualr particles. It is composed of colloidal silica treated with polyvinyle pyrolidine which is non toxic to the cells and dose not penetrate biological membrane [13]. The result of the present study demonstrated that one – step Percoll gradient procedure can be used successfully to separate MSCs from BM.

Mesenchymal stem cells was first described in 1970 by [14] who discovered that MSCs adhered to tissue culture plates resembled fibroblasts in morphology and grew in the form of colony. These characteristics have been identified in our culture of MSCs. So, after three days of culture, MSCs appeared sparsely and began to proliferate in culture medium, and after seven days cultivation numerous fibroblast like- cells were observed and gradually grow to form small individual colonies displaying fibroblast – like morphology. This finding also was recorded by [15, 16, 17], their finding showed that after seven days in the primary culture, numerous fibroblast –like cells began to proliferate and formed small individual colonies. Within the time of culture these small colonies continued to proliferate, and many cells displaying fibroblast – like morphology with short and long processes migrating from these colonies.

The result of the present study showed that, in the end of the second week MSCs formed the monolayer of adherent cells and this layer was expanded by two passages. Also our results correspond to other results such as [4,12] who reported the formation of the MSCs monolayer approximately in the second week of primary culture.

Immunophenotypic analysis of mesenchymal stem cells

Most specific surface markers have been found in adequate as a means to identify stem cells because the putative markers may also be found on non stem cells, and special (particular) markers may be only expressed on a stem cell at a certain stage or under certain conditions such as CD34 on HSCs. Within the BM , a simplified distinction is between CD34+HSCs, which are precursors of blood and endothelial cells, and CD34-MSCs, which are precursors of stromal cells, including fibroblasts [18,19].

In this study, we used the surface antigen CD11b, CD34, CD71, and CD90, to determine the phenotypic analysis of rat MSCs.

Under inverted microscope, the immunophenotypic analysis of rat MSCs showed that most of cultured adherent cells were positive response to CD71 and CD90. These results also observed by [11,12] who demonstrate that the BMCs showed a high percentage of CD71 and CD90 positive cells by immunostaining. This result indicate that these cells are primarily of mesenchymal origin.

According to this result, we noted that the most majority of cells in the rat MSCs cultures express CD71 and CD90 consistently with their undifferentiated state. These cells were stained with brown color DAB reaction, and the most majority of adherent cells are negative and not expressed for CD11b and CD34 markers , these cells stained with blue color. This observation are in agreement with other studies as in [11,20] who suggested that the cell surface markers CD11b and CD34 associated with lymphohematopoietic cells. Also the results of Nadri et al., [7] to the CD44, Sca-1 and CD 90 cell surface markers, and these cells are negative for the hematopoietic surfac3e marker such as

CD 34, CD 11b, CD 45, CD 31, CD 106, CD 117 and CD 135.

In conclusion , there was no evidence for the presence of hematopoietic cells in cultures which already removed by changing medium, that indicate the major population of adherent cells are MSCs.

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پوخته

شیکردنەوەی دیاری کردنی جۆری بەرگری لە خانە دروستکەرە گۆشتیەکانی مۆخی ئێسکی جورجی سپی

باکگراوند و مەبەستی لێکۆلینەوە: خانەی دروستکەرە گۆشتیەکان خانەی فرەدروستکەرن کە لە مۆخی ئێسک دەبینرێن و توانای دووبارە نوێبوونەوەیان هەیە, دەتوانن ژمارەیەکی زۆر لە خلنە جیاکەرەوەکان دروست بکەن و دووبارەنوێبوونەوەی شانەکان. ئامانج لەم لێکۆلینەوەیە بریتییە لە جیاکردنەوەو چاندنی (BM-MSCs) لە جورجی سپی و دیاری کردنی سروشتی دیاری کردنی جۆری لە (MSCs)ی جورج.

ڕێگای لێکۆلینەوەکە: مۆخی ئێسک لە جورجی سپی کۆکرایەوە و جیاکرایەوە بەبەکارهێنانی سەنترفیوجی پلەبەندی, دواتر خانە تاک ناوکەکان لەسەر میدیای گەشەیی (IMDM)و 10٪ سیرەمی گوڵکی بۆزیادکرا, دواتر ئەم کەلچەرانە لەژێر پلەی 37سo و 5٪ گازی دووەم ئۆکسیدی کاربۆن گەشەی پێکرا. دواتر شیکردنەوەی بەرگری ڕووکەشی بۆ ئەم خانانە ئەنجام درا بەبەکارهێنانی (CD90, CD71, CD34و CD11b).

ئەنجام: دوای 10–12 ڕۆژ لە دوای چاندنی سەرەتایی, خانە تاک ناوکەکان کە لە خانەی (MSCs) وەرگیراون بەخێرایی دووهێند دەبن و شێوەی خانەی فایبەری دەبن. لە کۆتایی هەفتەی دووەم خانە لکاوەکان یەک ڕیزیان دروست کرد کە زیادبوون دوای دووجار زیادکردن. ئەنجامی شیکردنەوەی دیاری کردنی جۆری بەرگری لە خانە دروستکەرەکان دەریخست کە زۆربەی خانەکان دەربرینی پۆزەتیڤیان هەبوو بۆ (CD90 و CD71) وە دەربرینی نێگەتیڤیان هەبوو بۆ (CD11bو CD34).

دەرئەنجام: ئەنجامەکانی ئەم لێکۆڵینەوەیە دەریخست کە زۆربەی خانە لکاوەکان بریتین لە (MSCs) وە ئەم خانانە دەربرینی پۆزەتیڤیان هەبوو بۆ (CD90 و CD71) وە دەربرینی نێگەتیڤیان هەبوو بۆ (CD11bو CD34).

الخلاصة

التحليل المظهري المناعي للخلايا الجذعية اللحمية المشتقة من نقي العظم في الجرذان البيض

خلفية واهداف البحث: تتواجد الخلايا الجذعية اللحمية ضمن البيئة الدقيقة لنقي العظم وهي خلايا جذعية بالغة متعددة القدرة لها القدرة على التكاثر والتجدد وتكوين أعداد كبيرة من الخلايا المتمايزة اضافة الى قابليتها على تجديد الانسجة التالفة .تهدف الدراسة الحالية الى عزل وزراعة الخلايا الجذعية لنقي عظم الجرذان والكشف عن الطبيعة المظهرية لهذه الخلايا بأستخدام معلمات (واسمات) مختلفة مثل:. CD 11b ,CD34 , CD90 and CD71

طرق البحث: تم جمع نقي العظم من ذكور الجرذان البيض اليافعة وفصلت بأستخدام النبذ المركزي المتدرج الكثافة وسحبت الخلايا الاحادية النواة من الطبقة الضبابية وزرعت في وسط زرعي مضاف اليه 10٪ مصل جنين العجل وحضنت المزارع بدرجة حرارية37مْ مع 5٪ غاز ثنائي اوكسيد الكاربون

النتائج: ظهرت الخلايا الجذعية اللحمية بشكل شبيه بالارومات الليفية وتتضاعف بشكل سريع بعد 10–12 يوم من الزرع الابتدائي .كونت الخلايا الملتصقة في نهاية الاسبوع الثاني من الزرع الابتدائي طبقة احادية من الخلايا وتوسعت هذه الطبقة بأستخدام زرعيين ثانويين. أظهرت نتائج التحليل المظهري المناعي للخلايا الجذعية اللحمية بانها ذات استجابة.CD11b , CD34 وذات استجابة سالبة لـ(CD71), (CD90موجبة لـ

الاستنتاجات: تشير نتائج الدراسة الحالية بان أكثر الخلايا الملتصقة هي خلايا جذعية لحمية وأظهرت هذه الخلايا استجابة موجبة للواسمات المتخصصة للخلايا الجذعية اللحمية لـ(CD71 CD90) , واستجابة سالبة للواسمات المتخصصة للخلايا الجذعية المكونة للدم .(CD34 , CD11b)

LEVELS OF SALIVARY BIOCHEMICAL'S IN PERIODONTITIS AND RELATED DISEASES

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ABSTRACT

Background and objectives: Periodontitis is one of the most wide spread disease in the world, and its prevalence in the center of Erbil city was high. Saliva was used as a noninvasive diagnostic fluid to measure chemical parameters released during oral diseases (especially sialic acid in periodontitis).
Method: This study included total sample of (286) individuals. They consisted of (149) males and (137) females. They included two age groups (18-44) years and (45-75) years. The individuals were distributed into 161 periodontitis patients, 59 patients of clinical controls, also 66 healthy individuals as controls. The levels of the following chemical biomarkers in both supernatant and sediment of saliva were measured: total sialic acid and its fractions (include; free sialic acid, lipid bound sialic acid and protein bound sialic acid), total calcium, total proteins.
Results: The levels of salivary biochemical parameters in periodontitis were higher than that of controls and clinical controls groups, except for some biochemical parameters in some groups of clinical controls. These groups were medicated mild inflammable group which showed non-significant difference with periodontitis in total proteins and total calcium. Also in gingivitis group there was a similarity in total calcium with periodontitis. In caries group protein bound sialic acid especially in sediment of saliva also showed a non-significant difference with periodontitis.
Conclusion: This study revealed that salivary total lipid bound sialic acid (TLSA) had significant role in raising total sialic acid in periodontitis and can be used with (TSA) in diagnosis of periodontitis of dentate. While the roles of total salivary; calcium (except in smoker) protein and protein bound sialic acid were no significant in periodontitis.

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Key words: salivary biochemical's, periodontal disease, simple caries

Periodontitis (periodontal disease) is a persistent bacterial infection causing chronic inflammation in periodontal tissues. It is characterized by the formation of pathologic periodontal pockets concomitantly with destruction of the periodontal ligament fibers attaching teeth to the alveolar bone and alveolar bone itself [1].

Periodontal diseases affect (5-30%) of the adult population. It is a multi-factorial influenced by genetics as well as by the environment [2] . It is one of the most widespread oral disease in the world, and is more prevalent in developing countries [3] particularly in rural area [4]. Prevalence of periodontal disease was very high (99.5%) in the center of Erbil city [5]. In Hawler city, [6] showed poor periodontal status in the age groups (25-29) years. Whole saliva serves as a reservoir for host-derived products (e.g. salivary gland components, gingival crevice fluid, and host enzymes) as well as exogenous components (e.g. oral microorganisms and microbial products). Considerable variation exist in the amount of salivary components among individuals and populations , and few well accepted

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normal value have been established for any specific marker[2]. Saliva could be used as a noninvasive diagnostic fluid to measure biomarkers released during disease initiation and progression such as periodontal infections. Saliva would also benefit from a faster screening of large sample size of subjects for epidemiological surveys [7].

Sialic acids are family of nine carbon acidic monosaccharide that occur naturally at the end of sugar chains attached to the surface of cells and soluble proteins [8]. Sialic acids occupy the interface between the host and commensally or pathogenic microorganisms. An important function of host sialic acid is to regulate innate immunity [9] . [10] Suggested that the amount of sialic acid in the saliva can be useful index of the severity of periodontal disease.

There is no sufficient studies in Kurdistan region about periodontitis showing relationship between the biomarker components of saliva (especially salivary sialic acid and its related parameters) and periodontitis, so this study was directed to investigate the levels of total sialic acid and different fractions of sialic acid (free, lipids & protein associated sialic acid), total salivary; calcium, proteins in periodontitis patients. Levels of the same biochemical parameters in controls (healthy periodontal patients) and clinical controls consist of small groups of patients with simple gingivitis, simple caries, medicated mild inflammable group and partial edentulous

METHODS

The present study was carried out in Erbil city-Hawler Medical University, College of Dentistry and Khanzad specialized dental center. The data collected through a personal interview in the dental teaching clinics during the period of 1st August 2008 up to 25th May 2009. The biochemical studies was done for all subject in the laboratory of basic science – College of Nursing.

The study population composed of three categories (161) cases (patients) attended the department of periodontics. They consist of ; 85 males and 76 females, their age range was (18-75) years and include patients with periodontitis, smoker periodontitis , and periodontitis patients with diabetes mellitus, and hypertension of blood.

Fifty nine (59) Clinical controls their age range was (19-75) years, and include (32) males and (27) females. They represent individuals who have simple caries and simple to moderate gingivitis, individuals with missing teeth more than 10 (partial edentulous), and medicated mild inflammable individuals.

Sixty six (66) controls, they represented individuals free from oral disease, which include (35) males and (34) females. Pregnant and lactating women, individual with viral, fungal infection were excluded. A structured questionnaire (interview forms) was used to collect data by asking the studied population about social and behavior factors include; age, sex, address, hereditary effect, diseases and use of medications

Saliva samples (prior to the clinical measurements) were collected from all subjects between 9 and 11 hours AM., to minimize the effect of diurnal variation on flow and composition. Spitting method [11] was used for collection un-stimulated whole saliva.

The time of collection was 5 minutes. The samples were stored at -200 C for one hour, then centrifuged immediately at (10000)g and at 40C for 20 minutes to obtain clear supernatant and are stored at -20C0 for analysis. The remaining sediment (precipitate) was washed with saline (7% pH 7.0), and centrifuged at 40C, 10000g for three minutes. The saline supernatant fraction was discarded and the wash procedure was repeated. The sediment was then re-suspended to the original volume

with saline (7% pH 7.0) and stored at -20C0 for analysis [12].

Biochemical analysis

Total and free sialic acid were measured with modified thiobarbituric acid method of Skoza and Mohos , 1976)[13]. Extraction and determination of lipid -bound sialic acid (LSA) (Masami, 1989) [14] Determination of sialic acid bound to total proteins (Shetty and Pattabriaman,, 2004)[15]. Determination of salivary total proteins Lowry method (Davidson, 2000)[16] . Determination of salivary total calcium (Moorehead and Briggs, 1974)[17].

RESULTS

This study included a total sample of (286) individuals. They consisted of (149) males and (137) females which represented 52% and 47% of the total sample size respectively. Their age ranged between (18-75) years. They were distributed into five cases; periodontitis 161(56.29%), simple gingivitis 25 (8.7%), simple caries 13 (4.5%), periodontitis with missing more than (10) teeth (partial edentulous) 13 (4.5%) and medicated mild inflammable periodontitis patients (medicated either with antibiotic or anti-inflammatory drugs) 8 (2.8%), and controls 66 (23.1%). All these groups with frequency of genders are shown in (Figure 1).

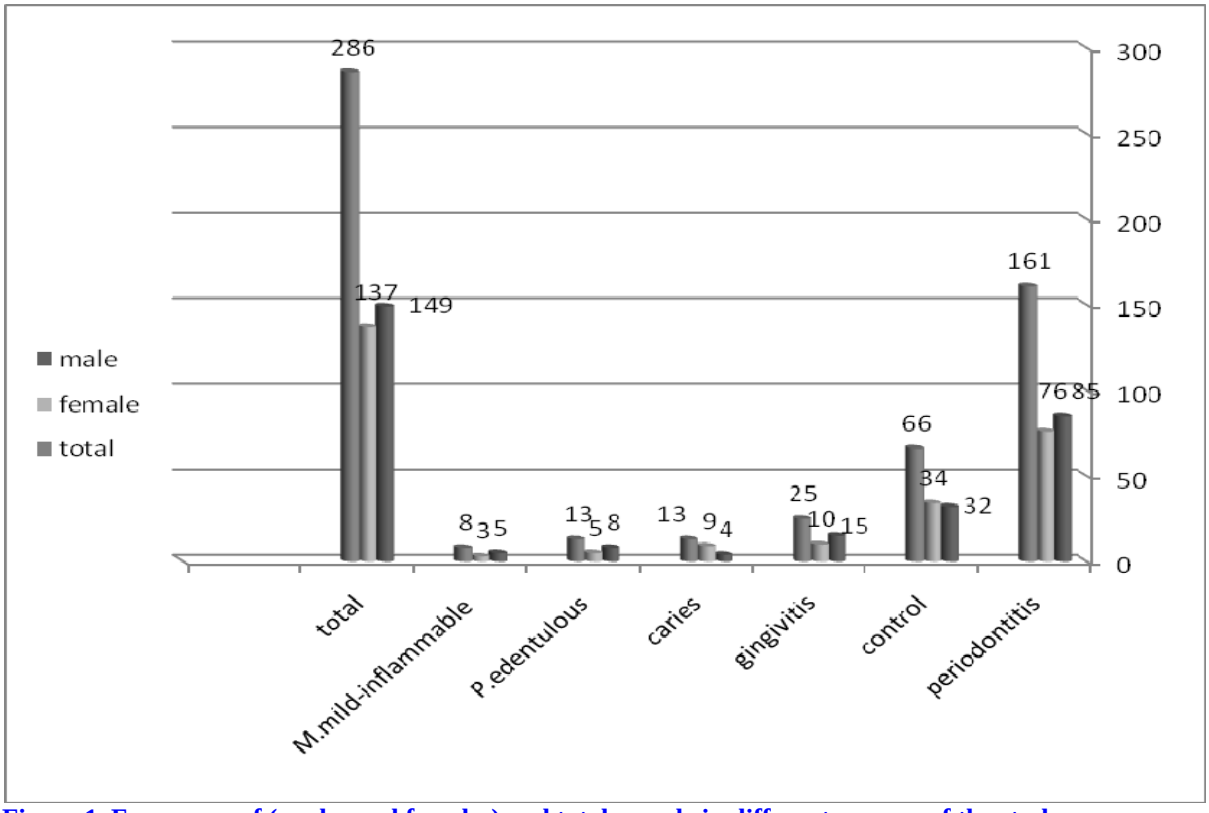

Figure 1. Frequency of (males and females) and total sample in different groups of the study.
P = Partial M= Medicated

The levels of total salivary; sialic acid, calcium and proteins in cases and control

Total sialic acid (TSA) , total calcium, and total protein were estimated in supernatant and sediment of saliva of all the cases; periodontitis, clinical control (included simple and moderate gingivitis, simple caries, partial edentulous periodontitis patients, medicated mild inflammable periodontitis patients) and control.

The statistical analysis showed significant ($P \leq 0.01$) difference for total sialic acid, total calcium, and total proteins among

groups, (Figure 2) . Multiple comparisons between groups was showed that the mean of total salivary sialic acid in periodontitis (160.58± 39.64 mg/l) is significantly (P≤0.01) higher than controls (76.34± 16.73 mg/l), gingivitis (106.75 ±12.42 mg/l), caries (124.39±15.69 mg/l) , Partial edentulous, (89.44±18.74 mg/l) , and medicated mild inflammable group (88.35±12.73 mg/l).

Significant (P≤0.01) difference in total salivary sialic acid was observed between the groups; caries (124.39±15.69 mg/l) with gingivitis (106.75 ±12.42 mg/l)) and controls (76.34± 16.73 mg/l) . While no significant difference was observed between groups ;partial edentulous (89.44±18.74 mg/l) with medicated mild-inflammable (88.35±12.73 mg/l) and controls (76.34± 16.73 mg/l) .

There was significant (P≤0.01) difference in total salivary sialic acid between groups of caries (124.39±15.69 mg/l) and partial edentulous (89.44±18.74 mg/l), and at (P≤0.05) between caries and medicated mild inflammable groups (88.35±12.73 mg/l). There was non-significant difference in total salivary sialic acid between gingivitis (106.75 ±12.42 mg/l) and groups (partial edentulous and medicated mild inflammable).

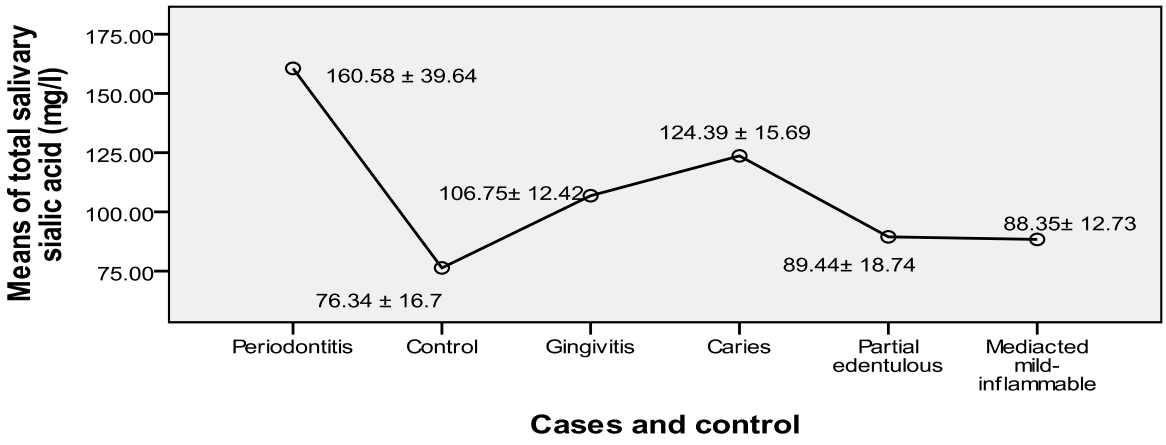

Figure 2. Means of total salivary sialic acid in controls and cases.

Multiple comparisons between groups for means of total salivary calcium was showed significant (P≤0.01) difference in periodontitis (9.97± 2.8 mg/dl) with controls (7.62± 2.4 mg/dl), with caries (7.84± 2.42 mg/dl), and with partial edentulous (7.95±2.14 mg/dl) at (P≤0.05). There was no significant difference in total salivary calcium between periodontitis and groups of (gingivitis 8.94± 2.82 mg/dl and medicated mild inflammable 8.98± 3.23 mg/dl). (Figure 3)

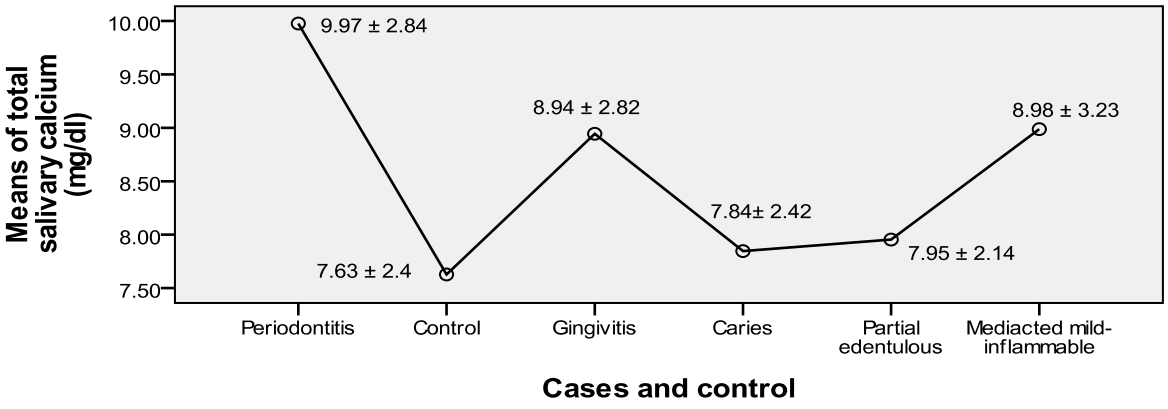

Figure 3.Means of total salivary calcium in cases and controls.

Multiple comparisons between groups for means of total salivary proteins, shows significant (P≤0.01)) difference of periodontitis (7.37±2.39 g/l) with controls (5.0±1.85 g/l), gingivitis (5.49± g/l) and partial edentulous (5.26± 2.09 g/l), and at (P≤0.05) with caries (5.8± 1.63 g/l) , but not with medicated mild inflammable (6.87± 2.22 g/l) group. (Figure 4)

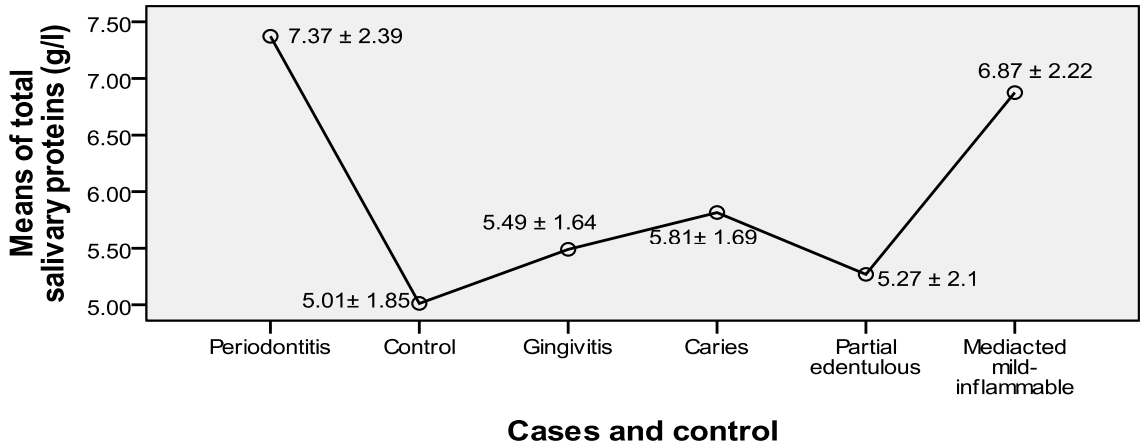

Figure 4. Means of total salivary proteins (g/l) in cases and controls.

Levels of salivary sialic acid fractions in cases and controls

Total salivary sialic acid (TSA) represents the sum of free sialic acid (FSA), lipid bound sialic acid(LSA) and protein bound sialic acid (PSA) in both supernatant and sediment fractions of saliva.

Statistical analysis of multiple comparisons, (Figure 5) shows significant (P≤ 0.01) difference between levels of total salivary free sialic acid (TFSA) in periodontitis (54.1± 18.28 mg/l) and (control 26.59 ± 11.96 mg/l). Also (TFSA) in periodontitis and cases; gingivitis (39.87± 15.09 mg/l) , caries (39.27 ± 12.44 mg/l) , partial edentulous (27.4 ±8.29 mg/l), and medicated mild inflammable (34.79 ± 14.49 mg/l) group. While (TFSA) in control had significant (P≤ 0.01) difference with caries and gingivitis but had no significant difference with groups of partial edentulous and medicated mild inflammable. Both gingivitis and caries were nearly similar in (TFSA) and had significant (P≤ 0.05) difference with group of partial edentulous. The (TFSA) in medicated mild inflammable group (34.79 ± 14.49 mg/l) had no significant difference with all cases and controls except periodontitis (54.1± 18.28 mg/l).

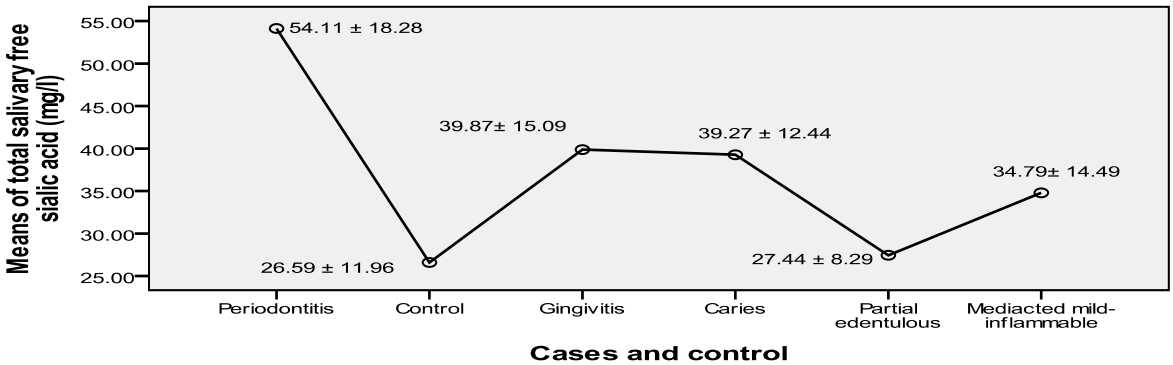

Figure 5 .Means of total salivary free sialic acid in cases and controls.

Statistical multiple comparisons in the levels of total salivary protein bound sialic acid (TPSA) showed significant (p≤ 0.01) difference in (TPSA) between periodontitis patients (40.95 ± 21.41 mg/l) and control (22.21± 7.96 mg/l). Also (TPSA) in periodontitis showed significant (p≤ 0.01) difference with groups; gingivitis, partial edentulous and medicated mild inflammable, (25.7 ± 10.51, 25.3 ± 11.5, 23.02 ± 12.86 mg/l) respectively except with caries group (40.07 ± 14.69 mg/l). Caries group had significantly (p≤ 0.01) higher level of (TPSA) comparing to controls. Also significantly (p≤ 0.05) higher level comparing to groups; gingivitis, partial edentulous and medicated mild inflammable. Gingivitis, partial edentulous and medicated mild inflammable groups had no significant difference between each other's and with controls in (TPSA) as shown in (figure 6).

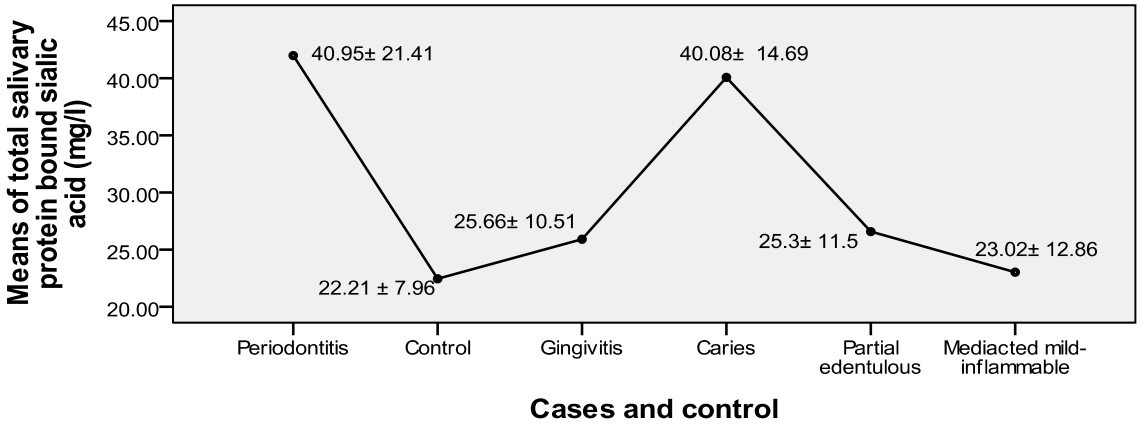

Figure 6. Means of total salivary protein bound sialic acid in cases and controls.

Levels of total salivary lipid bound sialic acid (TLSA) in periodontitis (65.52± 25.77 mg/l) had significant (P≤ 0.01) difference with controls (27.1± 9.35 mg/l) and also with (gingivitis, caries, partial edentulous and medicated mild inflammable) groups (41.22± 14.66 , 45.1± 7.63 , 36.7 ± 8.43 and 30.9± 8.59 mg/l) respectively . While (TLSA) in control had significant difference (P≤ 0.01) with other groups (gingivitis, caries, partial edentulous and medicated mild inflammable) that were not changed significantly, (Figure 7).

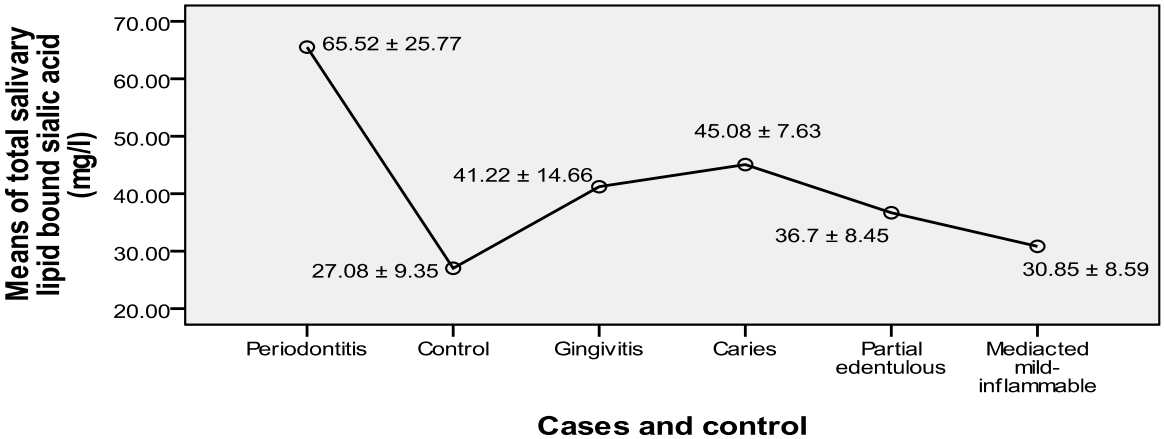

Figure 7 . Means of total salivary lipid bound sialic acid in cases and controls

DISCUSSION

Salivary biochemical parameters

Salivary glycoprotein plays an important role in the properties and functions of saliva. Interest in saliva as a diagnostic fluid has grown in recent years and biochemical analysis of saliva was not evaluated in detail in routine clinical laboratories. These prompted to estimate salivary biochemical parameters as marker of periodontitis.

Total Salivary sialic acid

In this study, total salivary sialic acid showed significant higher concentration in periodontitis patients comparing with controls . This was accompanied with that salivary sialic acid concentration in the periodontal group was significantly higher than that in healthy group [10] . This increase in total salivary sialic acid may be due to presence of certain Gram negative bacteria (periodpathogenic) adhered only to low molecular weight and free form of salivary mucin (MG2) throughout the sialic acid residues present on MG2 which may cause a decrease in (MG2) and an increase in sialic acid [18]. Also increase synthesis of mucin as scavenger of hydroxyl group throughout sialic acid was resulted from increase in lipid peroxidation in periodontal diseases [19] . Serum sialic acid concentration was elevated in patient with bacterial infection. This was due to the change in biosynthesis and post translational glycosylation process of the acute-phase glycoproteins in liver [20]. Elevation in sialic acid concentration during inflammation was resulted from elevation in richly sialylated acute-phase glycoproteins [21].
Significant difference was observed between periodontitis and clinical controls in the level of total salivary sialic acid. This result was similar to that of [22] who reported that the sialic acid concentration increased with the severity of inflammatory symptoms compared to a group of patients with cellulites and other diseases with relatively mild symptoms. [10] suggested that total salivary sialic acid was a useful parameter for determining the severity and course of periodontal diseases. [23] indicated that total salivary sialic acid levels in Down's syndrome were higher than in controls of similar caries indices. While [24] found significant differences in sialic acid content of mixed saliva between healthy and an active caries groups.

Total salivary calcium

The result showed significant higher concentration of total salivary calcium in periodontitis patients than in control and simple caries. This was in agreement with [25] who detected a higher concentration of calcium in peridontitis than healthy subjects. Positive correlation was observed between high salivary calcium content and periodontitis, suggesting that a person with high calcium content of plaque and saliva might be susceptible both to calculus formation and periodontitis. [26] study showed that elevation in calcium concentration of saliva is a characteristic of periodontitis. High salivary calcium in periodontitis may be due to calculus (calcified plaque which is resulted from deposition of calcium and phosphorus) that build up along gingival and cause inflammation [27].
Low total salivary calcium in simple caries may be due to low caries experience, and agree with[28] who reported that caries is demineralization of the inorganic portion and destruction of the organic substance of the tooth and the concentration of salivary calcium increased with increase severity of caries experience (DMFS).
Total salivary calcium in gingivitis and medicated mild inflammable groups had a high total salivary calcium and mild periodontal disease and they include more men than women .In gingivitis may be due

to genders (men has higher rate of salivary secretion) and the stage of gingivitis, Percentage of men were (60%) in gingivitis group. This was in agreement with [25] who found more elevated salivary calcium concentration in men than women, men showed more bleeding on probing and lower (DMFS) – scores of caries and concluded that salivary calcium may be important with regard to both dental and gingival health. While medicated mild inflammable group were (80%) smoker men, may affect the levels of salivary calcium. This was similar to [26, 29] who found higher salivary calcium concentration in heavy smokers than in non smokers.

Total salivary proteins

The total salivary protein was significantly higher in periodontitis comparing with controls and clinical controls except for the medicated mild inflammable group. This increase may be due to several mechanisms that take place in periodontal diseases (mainly due to bacteria products and tissues break down) . The result was in agreement with that of [29] who found high concentration of total proteins in periodontitis and gingivitis compare to controls, [30] identified that subject with poor gingival health had higher concentration of total protein than those with no need for periodontal treatment. [31] Study indicated that subject with periodontitis showed enhanced amylase (major protein of parotid gland) concentration in saliva. An increased level of salivary IgA and IgG reflect oral inflammation [32]. [33] Observed elevated levels of total salivary proteins and sugars in oral cancer compared to those of healthy controls.

Bacterial pathogens such as Porphyromonas gingivallis has the capacity to activate host defense mechanisms that break down the epithelia and other structures of the gum and inactivate of repair system in individuals with periodontal disease with a lack of antioxidant defense [34] and they found increase in concentration of salivary protein carbonyl (as index of oxidative injury) in periodontitis. [35] Stated that bacteria- host interaction cause stress and production of reactive oxygen species which cause degradation of host tissues and accumulation of bacteria products.[36] study showed that a number of enzymes, tissue breakdown products and inflammatory mediators are released from host cells and tissues during the development and progression of periodontitis infections. [37] indicated that infection of tissues surrounding the teeth is associated with elevated level of C-reactive proteins (CRP).

In recent study total protein in simple inflammable cases (gingivitis, caries, partial edentulous) was similar to control group. This may be to the decrease of protective protein. This result was similar to [38] who proposed that decrease of protective protein , accompanied with an increase in antimicrobial proteins which include proline-rich proteins, lysozyme, lactoferrin, sialoperoxidase, agglutinins and histidine, as well as secretary immunoglobulin A (sIgA) and immunoglobuulins G (IgG) and (IgM) and it is equilibrated with saliva [27] .

Non-significant difference in total salivary proteins in medicated mild inflammable group with periodontitis in contrast to total sialic acid which was low (88.35±12.7 mg/l) comparing to periodontitis (160.58±39.64 mg/l). This may be due to higher percentage (80%) of men smokers in this group and high salivary calcium. The result in line with that of [32] who concluded that smoking habit cause low inflammatory markers. Low antioxidant defense capacity in smokers which cause high tissue injury [34]. Smoking and nicotine increase an over production of immune factors (interleukins), which in excess are harmful to cells and tissues [39].

Levels of biochemical parameters in partial edentulous group

The (84.6%) of this group were in old age group (45-75) years and their results were not differ from controls in total salivary sialic acid, calcium and proteins. This may be due to that less inflammation will be occurred in this group. The result was in line with that of [32] who found that the intensity of periodontal inflammation has been associated with the number of teeth affected.. The study also indicated that subjects with fewer remaining teeth had less evidence of periodontitis than those with more teeth [40] and they suggested that these subjects had received therapy with selective extractions of teeth affected by periodontitis.

Levels of salivary sialic acid fractions in cases and controls

Total free sialic acid

Higher significant concentration of total free sialic acid (TFSA) in periodontitis compare to other cases and controls. Mild inflammable groups (Gingivitis , caries, medicated mild inflammable were differ significantly from non-inflammable groups (controls and partial edentulous). This agree with [31, 44] who found high levels of (FSA) in oral cancer and in alcoholic patients respectively.

In this study high (FSA) in periodontitis may be due to large number of oral bacteria which produce neuraminidase that cleaves terminal sialic acid residues from carbohydrate moieties of salivary glycoproteins and abolishes aggregation and removing bacteria from the oral cavity [42].

Control group which had good oral hygiene had higher level of (TFSA) (26.59 ± 11.96 mg/l). This was in agreement with [43] who suggested that healthy subjects with good oral hygiene had significantly higher levels of free sialic acid on their teeth surface than gingivitis subjects. [44] Who indicated that sialic acid residues in sub maxillary gland mucin is an essential moiety to scavenge (OH) group and release of free sialic acid.[45] Who found that free sialic acid may be an alternative oxidative stress marker in healthy athletes

Medicated mild inflammable group not differs from gingivitis and caries. This result may be due oxidative stress in this smoker group. This result was in agreement with [46] who found positive correlation of (TSA) with antioxidant enzymes. Prolonged stimulation by nicotine also cause increase in total proteins and decrease in glycosylation of salivary glycoprotein [11] .

Total protein bound sialic acid

The total protein bound sialic acid (TPSA) levels in periodontitis, and caries were significantly higher comparing to control , gingivitis , partial edentulous , and medicated mild inflammable group . This result agree with [21] who found richly glycated glycoproteins in periodontitis. Also in line with [33] who estimated elevated levels of salivary (PSA) in oral cancer. Whereas low level of (PSA) in gingivitis was in consistence with [15] who found reduced level of protein bound sugar in gingivitis and periodontitis.

No significant difference between caries and periodontitis was in agreement with [47] who stated that the high molecular-weight of glycoprotein (which was characterized by a high content of carbohydrates, associated lipids and covalently bound fatty acids) was predominant in caries susceptible mucus and aids adhesion of bacteria , whereas the low molecular glycoprotein (which was richer in protein and contained lesser amount of associated lipids and covalently bound fatty acids and more sialic acid) predominant in caries resistant mucus and aid aggregation and removing of microbes

Homogeneity between periodontitis and caries in (TPSA) especially in sediment fraction of saliva may be due to bacteria products and their cell walls. The cell wall

of Gram positive bacteria in caries rich in piptidoglycan while cell wall of Gram negative bacteria in periodontoitis have only one or a few layers of piptidoglycan but they possess an outer membrane consist of lipopolsaccharide. This structure reflected in high levels of (PSA) (18.77± 14.26) and (LSA) (21.14± 14.2) in sediments of saliva of periodontitis, while in caries only (PSA) (14.5 ±6.4) in sediment was significantly higher comparing to other groups. This was in agreement with [48] who mentioned that removal of terminal negatively charged sialic acid by neuraminidase could cause precipitation of glycoprotein out of saliva and their protein residues incorporated onto the surface of the developing plaque (bacterial bio-film) and the bacteria use sugars derived from glycoprotein. [9] Reported that sialic acids also were excellent sources of carbon, nitrogen, energy, and precursors of cell wall biosynthesis.

High levels of total (PSA) in caries may also be due to destruction of teeth tissues, (dentin and cementum) which were high in sialoprotein[49] by acid forming bacteria in dental plaque [35] .

Total lipid bound sialic acid

The highest significant value of total lipid sialic acid (TLSA), was found in periodontitis patients (represent 42.7% of TSA) and the lowest was found in control which was also different significantly from the cases.

The Lipid bound sialic acid levels in gingivitis, partial edentulous group, and medicated mild inflammable group were significantly lower than periodontitis. This may be due to unsialylated lipopolysaccharide strain of bacteria in these patients. This result was accompanied with that of [50] who concluded that incorporation of N-acetylnuraminic acid into F. bacterium nucletum lipopolysaccharide (most dominant of oral bacteria) can hinder the function of the host defenses via disruption of the complement pathway. [9] Mentioned that microbes decorated their surfaces with sialylated oligosaccharides that mimic those of the host sialic acid which regulate innate immunity. [51] Found that lipo-polsaccharide enhance endotoxin level, oxidative stress (reactive oxygen species production), tissues inhibitor of metalloproteinase-1, aspartate aminotransferase, and cytokines production.

In caries group (LSA) was significantly lower than periodontitis and agree with[38] who reported that there were no difference in lipid peroxidation between groups with caries and group without caries and stated that lipid peroxidation induced mucin synthesis and total sialic acid increases in saliva. [52] research showed that total sialic acid increased with increasing lipid peroxidation and it showed that the antioxidant enzymes were involved in protecting membrane bound lipid as well as membrane bound sialic acid from exogenous reactive oxygen species . [53] Stated that lipid peroxidation increased in periodontitis and some systemic diseases such as diabetes mellitus, etc.

Marked elevation of serum sialic acid concentration (TSA and /or LSA) that correlate with the clinical activity of a disease have been documented in many malignancies [54]. [55] reported that in a patient having primary gastric cancer whose total sialic acid concentration has not increased yet (LSA) concentration of the patient has significantly increased.

CONCLUSIONS

High significant role of salivary total lipid bound sialic acid (TLSA) in raising total sialic acid in periodontitis demonstrate sialylation of lipopolysaccharide of gram negative bacteria and can be used with (TSA) in diagnosis of periodontitis of dentate (more than 21 teeth) patients.

Non significant role of total salivary; calcium (except in smoker) , protein and protein bound sialic acid in diagnosis of periodontitis , improved by homogeneity ; in the levels of total calcium between periodontitis and gingivitis , in the levels of total; proteins and calcium between periodontitis and medicated mild inflammable group , and in the (TPSA) between periodontitis and caries .

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پوختە

توێژینەوەی ماددە کیمیاویەکانی لیکاو لە نەخۆشی هەوکردنی پەرەسەندووی پووک و نەخۆشییە پەیوەندی دارەکانی دەم

هەوکردنی پەرەسەندووی پووک کە لە نەخۆشییە بلاّوەکانی جیهانە و بریتیتیە لە تێك شکانی ریشاڵە پاڵپشتەکانی ددان ,
بۆ ئەنجامدانی ئەم توێژینەوەیە نموونەی لیك لە (286) کەس وەرگیرا کە تەمەنیان لە نێوان (18–75) ساڵ دابو وە هەردوو رەگەزیش دەگرێتەوە خۆ (149 نێر) وە (137 مێ). ئەم کۆمەلە نموونەیە پێکهاتبوون لە (161) نەخۆشی هەوکردنی پەرەسەندووی پووك,وە (59) نەخۆشی هەوکردنی سادەی پووك و ددان کە بریتی بوو لە 25 هەوکردنی ئاسایی پووك ,13 تووشبوو بە (تسوس)ی سادەی ددان, 13 یان ددانەکانیان نا کامل بوو (کەمتر لە بیست و دوو ددان) وە 8 یان دەرمانیان وەرگرتبوو وە ئەوەی ماوەتەوەش (66) کەس بریتیبوون لە کۆنترۆل

لە توێژینەوەکەدا پێوانەی ماددە کیمیاویەکانی لیکاو بەم شێوەیە کراوە: ترشی سیالیك گشتی , ترشی سیالیك ئازاد , ترشیسیالیك بەند بوو بە پرۆتی ترشی سیالیك بەند بوو بە چەوری ,کالسیۆمی گشتی,پرۆتینی گشتی.

ئەنجامی توێژینەوەکە دەریان خست کە بەرزترین ئاستی ماددە کیمیاویەکان لە نەخۆشی هەوکردنی پەرەسەندووی پووك بەبەراورد لەگەل نەخۆشی هەوکردنی سادەی ددان وە کۆمەلەی کۆنترۆل , تەنها چەند کۆمەلەیك لە هەندیك لە ماددە کیمیاویەکان .ئەمانەش بریتیبوون لە کۆمەلەی نەخۆشی هەوکردنی سادە لە ئەنجامی بە کارهێنانی دەرمان کە ئاستی بەرزی هەبوو لە پرۆتین و کالسیۆم, کۆمەلەی نەخۆشی هەوکردنی ئاسایی پووکی ددان کە ئاستی بەرزی هەبوو لە کالسیۆم هەروەها کۆمەلەی نەخۆشی (تسوس)ی سادەی ددان کە ئاستی بەرزی هێنا لە پرۆتینی بەندبوو بە ترشی سیالیك.

ترشی سیالیك بەند بوو بە چەوری بەرزترین ئاست و بەبەراورد لەگەل نەخۆشی هەوکردنی سادەی ددان وە کۆمەلەی کۆنترۆل . جیاوازی نامەعنەوی بەدی کرا لە نێوان نەخۆشەکان کە ددانییان لە (22) ددان کەمترە وە کۆمەلەی کۆنترۆل , ئەمەش دەگەڕێتەوە بۆ کەمی هەوکردنی پووك کە بەستراوە بە ژمارەی ددانەکان ,

ئەنجامی توێژینەوەکە گرنکی ترشی سیالیك وە ترشی سیالیك بەند بوو بە چەوری وەك پێوەرێك بۆ دەست نیشان کردنی نەخۆشی هەوکردنی پەرەسەندووی پووك دەردەخات .

الخلاصة

مستويات المؤشرات الكيميائية الحيوية اللعابية في periodontits والأمراض ذات الصلة

Periodontits هي واحدة من الأمراض الأكثر انتشار في العالم، وانتشاره في وسط مدينة أربيل كانت مرتفعة. تم استخدام اللعاب كسائل تشخيصي لقياس المعلمات الكيميائية الصادرة اثناء الإصابة بأمراض الفم (الحامض اللعابي خاصة في periodontits).

منهجية البحث: وشملت هذه الدراسة عينة مكونة من 286 فرداً، مألفة من 149 ذكرا و 138 انثى. وتشمل الفئات العمرية 18 – 44 سنة و 45 – 75 عاما. مكونة من 161 من المرضى المصابين بال periodontits، و59 مريضا من الضوابط السريرية، و 66 فردا من الضوابط الاصحاء. تم قياس مستوى المؤشرات الكيميائية الحيوية في كل من supernatants والرواسب من اللعاب: إجمالي الحامض اللعابي وجزيئاته (وتشمل، حامض الرسوم اللعابي، والحامض الدهني اللعابي و ملازمة البروتين والحامض اللعابي)، الكالسيوم الكلي والبروتينات الكلية.

النتائج: كانت مستويات المؤشرات الكيميائية الحيوية اللعابية في periodontits أعلى من الضوابط ومجموعات المراقبة السريرية، فيما عدا بعض القياسات البيوكيميائية في بعض مجموعات من عناصر التحكم السريرية. وكانت هذه المجموعات مجموعة العلاج قابلة للالتهاب والتي أظهرت عدم فرق غير معتد بها احصائيا الفرق مع periodontits في البروتينات الكلية والكالسيوم الكلي. أيضا، هناك تشابه في الكالسيوم الكلي بين مجموعة التهاب اللثة و periodontits. في مجموعة التسوس ملازمة الحامض البروتيني اللعابي خاصة في الراسب من اللعاب كما أظهرت بان الفارق غير معتد مع periodontits.