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XMN I POLYMORPHISM IN B-THALASSEMIC PATIENTS IN THE DUHOK REGION –IRAQ

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ABSTRACT

Background and objectives Several genetic modifiers have been implicated in phenotypic variations in β-thalassemia(thal) syndromes. Among the frequently implicated ones in the Eastern Mediterranean is Xmn I polymorphism. No study has addressed the relative contribution of this polymorphism to the phenotypic variation of Iraqi β-thalassemia patients.
Methods A total of 107 symptomatic β-thal patients (52 thal Intermedia and 55 thal major) were enrolled. Their clinical records were reviewed. All had full blood counts as well as HbA2 and F quantitation by high performance liquid chromatography performed. The presence of Xmn I polymorphism was documented by PCR-RFLP based method.
Results The homozygous XmnI (+/+) status was found in 19.2% and 1.8% of the thal intermedia and major patients respectively (p=0.0028). Heterozygous state Xmn I (+/-) was found in 32.7 and 21.8% respectively. Overall and among the 107 patients enrolled the (+/+), (+/-) and the (-/-) were associated with median annual transfusion rates of 0, 3.5 and 11 respectively, with significantly lower requirements of those with the (+/+) and (+/-) when compared to non-carriers (p=.004 and 0.02 respectively). The highest Hb F levels were found among those with the (+/+) and the least in the (-/-), a finding which was significant (p=0.029).
Conclusions Xmn I polymorphism is an important genetic modulator of severity of β-thal among Iraqi Kurds, and is associated with higher Hb F levels than non-carriers. Further genetic modulators need to be investigated to determine their relative contributions to the phenotypic variation of this important genetic disease.

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Key words: B-thalassemia, Duhok, XmnI polymorphism, Iraq

β-thalassemia (thal) is an autosomal recessive genetic disease, which is among the most frequently encountered inherited hematological disorders in Iraqis, including those from Duhok province in the extreme north.[1-4] β-thalassemia may present as a severe transfusion dependent thalassemia major phenotype (with

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homozygous or compound heterozygous genotypes), or a relatively asymptomatic thalassemia minor (with heterozygous genotype) or an intermedia phenotype with intermediate clinical severity and a variable genotype.[5] Several genetic factors have been implicated in modulating the severity of symptomatic β-thalassemia, including the type of β-gene mutations, the concomitant α-gene status and increased γ-gene production.[6] Among the most important genetic factors involved in the latter, is a mutation causing a C to T base pair substitution at the -158 position in promoter region of the $^{G}\gamma$-globin gene. This mutation leads to the creation of a digestion site for the restriction enzyme *Xmn* I (*Xmn* I polymorphism).[7] Although *Xmn* I polymorphism contribution to the phenotypic variation in β-thalassemias has been investigated and documented in several populations, no such study was reported among Iraqis. The aim of the current study is to address the latter issue through studying a group of β-thalassemia major and intermedia patients.

METHODS

A total of 107 cases of symptomatic β-thalassemia patients were recruited from the thalassemia care center in Duhok. They included 55 patients with thal major (TM) and 52 with thal intermedia (TI). A careful review of the clinical and laboratory records of all enrolled patients was undertaken. The diagnoses of thal major or intermedia were based on the results of initial investigations at diagnosis as well as clincial follow up and transfusion dependence.

A sample of 7.5 mL of venous blood was taken and distributed between 3 EDTA tubes. One sample was used to perform a full blood count via an electronic hematology analyzer (Beckman coulter – USA), another to perform high performance liquid chromatography (HPLC) (VARIANT^{TM}; Bio-Rad Laboratories, Hercules, CA, USA) for quantitation of Hb A, A2 and F, while the third sample tube was stored at -20C and was thereafter used for DNA extraction using a phenol-Chloroform method.[8]

The extracted DNA was then amplified for a 650 bp sequence in the promoter region of the $^{G}\gamma$-globin gene. This amplification was performed using an AB 2720 thermocycler (Applied Biosystems-USA) applying the following primers: Forward 5' AAC TGT TGC TTT ATA GGA TTT T3' and Reverse 5' AGG AGC TTA TTG ATA ACT CAG AC 3'. The PCR program used started with an initial denaturation for 2 min at 94^{O}C, followed by 30 cycles of denaturation at 95^{O}C for 1 min, annealing 60^{O}C for 1 min, and extension 72^{O}C for 1.5 min. Thereafter, a final extension for 5 minutes at 72^{O}C.[8]

The 650 bp amplicon was then digested with the enzyme *Xmn* I according to the manufacturer's instructions (Promega-USA), and the digestion products were run on a 2% agarose gel and visualized using ultraviolet transilluminator after staining with ethidium bromide.

This study was approved by the ethical committee at the Scientific Research Center, University of Duhok.

Statistical analysis utilized the SPSS software program. Chi square (with Yates correction) and Mann Whitney U test were used when applicable. $P < 0.05$ was considered significant.

RESULTS

The results of molecular studies showed that out of the 52 patients with thal intermedia: 10 patients (19.2%) were found to be homozygous for *Xmn* I (+/+), compared to only 1/55 in the thal major group (1.8%); while 17/52 (32.7%) of thal intermedia and 12/55 (21.8%) of thal major were heterozygous for it (+/-). Overall, those who were carriers of the *Xmn* I polymorphism (whether +/+ and +/) and particularly those homozygous for it

(+/+) were significantly more frequent among thal intermedia group (p=0.0048 and 0.0028 respectively). Figure 1 shows the RCR-RFLP results.

The results also revealed that out of the total 104 chromosomes in the thal intermedia group 37 (35.6%) had the *Xmn* I polymorphism, compared to 14 (12.7%) of the 110 thal major chromosomes. A finding which was highly significant (*p=0.0002*).

Median transfusion requirements/year were 0, 3.5 and 11, in those with *Xmn* I (+/+), (+/-) and (-/-) categories respectively regardless whether they were in thal major or intermedia categories (with significantly lower requirements in (+/+) and (+/-) when compared to (-/-) with *p* of 0.004 and 0.020 respectively).

When median hemoglobin concentration (Hb), Hb F and Hb A2 were compared between those with the *Xmn* I polymorphism and those without it in the 50 thal intermedia patients who did not receive any transfusions in the past 2 months, it was found that there were significant higher Hbs in those with (+/+) when compared with the (-/-) (p=0.044) but no significant differences between (+/-) and the (-/-) genotypes (p=0.072). Furthermore, Hb F was significantly higher in those with (+/+) and (+/-) when compared to those with (-/-) genotype (p=0.029 and 0.022 respectively). Hb A2 on the other hand, was significantly lower in those with (+/+) and (+/-) compared to those without the polymorphism (p=0.006 and 0.009 respectively). Table 1 outlines the median Hb, HbF and Hb A2 in association with *Xmn* I polymorphism.

Table 1. Median Hb, Hb A2 and F levels in relevance to the Xmn I polymorphism in 50 Thal Intermedia patients from Duhok

Parameter	*Xmn*I status (No.)		
	+/+	+/-	-/-
	9	17	24
Hb (g/dl)	9.9	8.2	8.6
Hb A2 (%)	3.5	3.8	5.95
Hb F (%)	91.7	45.5	16.25

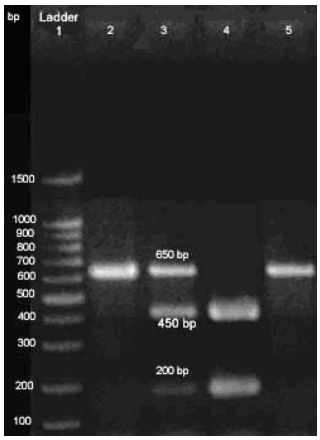

Figure 1. Represent 2% gel electrophoresis for PCR-RFLP analysis of Xmn 1 polymorphism. Lane 1: 100 bp DNA ladder; Lane 2: 650 bp fragment from a patient with Xmn I (-/-) genotype; Lanes 3: 650 bp, 450 bp and 200 bp fragments from patients heterozygous for the Xmn I (+/-) genotype; Lanes 4: 450 bp and 200 bp digested fragments from a patient with Xmn I (+/+) genotype; Lane 5: Undigested 650 bp amplified sequence

DISCUSSION

Several factors have been implicated in modulating the severity of β-thalassemia, including those which lead to increase in γ-globin chain production. *Xmn* I polymorphism has been reported to be associated with 3-11 folds increase in $^{G}\gamma$-globin chain production, by increasing the rate of the transcription of the gene, in conditions characterized by hemopoietic stress.[7,9,10] The subsequent increase in cellular Hb F content offers a selective survival advantage to the cells and thus ameliorating the disease.[7,9] Its contribution to the molecular basis of thalassemia intermedia varies in different populations, with the highest rates reported among Iranian and the least among the Chinese.[11,12]

The findings of the current study show that among Kurdish thalassemic patients, *Xmn* I polymorphism is more frequently encountered in those with thal intermedia when compared to those with thal major, and that homozygosity (+/+) was significantly higher among TI. This

indicates that *Xmn* I polymorphism is an important modulator of thal severity among the Iraqi Kurds. This is strongly supported by the findings that the median transfusions per year decreased from a median of 11/ year in those with *Xmn* I (-/-), to 3.5/year in the *Xmn* I (+/-) to zero/year for the (+/+) category, an observation which was found to be significant. However, in patients with thal intermedia, hemoglobin was only significantly higher in those with *Xmn* I (+/+), but not in those with (+/-) genotypes, versus the (-/-) genotype. Previous investigators have suggested that significant amelioration of thalassemia was more likely in (+/+) than (+/) genotypes.[6]

When compared to other studies on thalassemia intermedia, our 19.2% *Xmn* I (+/+) figure is near to those reported from Lebanon at 21.9% by Qatanani and coworkers (2000), Mediterraneans and Asian-Indians by Ho and coworkers (1998) and within the range given for Indians of 12.5-27.4%, but was much less than the high figure of 40% reported by Neishabury and colleagues (2008) among Iranian TI patients.[11-16] In all the above mentioned populations it appears that *Xmn* I (+/+) polymorphism is an important contributor to the molecular basis of TI, which is in contrast to almost absent role of this polymorphism among the Chinese TI, where (+/+) polymorphism is seen in <1% of TI patients.[12]

Hemoglobin, Hb F and A2 could only be compared in thal intermedia patients (50/52) who did not receive a recent transfusion. Interestingly and to further support the role of *Xmn* I polymorphism as a modulator of Hb F, it was shown that Hb F was significantly higher in those with the polymorphism whether in homo or heterozygous state than non-carriers. However, the remarkable variation in Hb F levels in those with *Xmn* I polymorphism and those without it, indicates that those with (-/-) are more likely to have another major ameliorating factor which is likely to be the inheritance of mild β-thal mutations (homozygous or compound heterozygous) or heterozygosity to such mutations. Both latter categories are associated with lower Hb F levels.[5]

In conclusion, this study has shown that *Xmn* I polymorphism is an important β-thal genetic modulator in Iraqi Kurdish thal patients, and that this polymorphism most likely exerts its effect through increasing Hb F. However, further studies on other genetic modulators of the phenotype of symptomatic β-thal are warranted. Among those requiring further scrutiny are types of the β-thal mutations and concomitant α-thal.[5,9,14]

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پوختە

مشەشێوەییا Xmn I ل نەخوشێن دەریایێ سپی ناوەراست ل جورێ بێتا ل دهۆکێ

پێشەکی و ئارمانج: گەلەك گهورکەرێن زکماکی یێن هاتینە نیشانکرن بو توشبوونێ ب جورەیێن شێوەیی یێن نەخوشیا تالاسیمیا بێتا یا ب نیشان. ژ یێن مشە هاتینە نیشانکرن ل روژهەلاتا ناڤەراست مشەشێوەییا Xmn I. چ ڤەکولینا بابەتێ گرێدانا ڤێ مشەشێوەییێ دگەل جورەیێن شێوەیی یێن نەخوشێن تالاسیمیا بێتا یێن عیراقی نە وەرگرتییە.

ریکێن ڤەکولینێ: سەرجەمێ 107 نەخوشێن تالاسیمیا بێتا هاتنە وەرگرتن دڤێ ڤەکولینێ دا (52 ژ جورێ تالاسیمیا ناڤین و 55 یێن تالاسیمیا مەزن). پێداچوونەك د تومارێن وان دا هاتە کرن و تاقیکرنا تەمام یا خوینێ و هیموگلوبینێ A و A2 و F بو هاتە کرن بریکا HPLC و DNA هاتە ڤاڤارتن. ئەڤ DNA هاتە بکارئینان بو دەستنیشانکرنا مشەشێوەییا Xmn I بریکا PCR-RFLP.

ئەنجام: هاتە دیتن کو Xmn I یێ وەك هەڤ (+/+) هەبوو ل دەف 19.2٪ و 1.8٪ ژنەخوشێن تالاسیمیا ناڤین و تالاسیمیا مەزن لدویف ئێك (p= 0.0028). حالەتێ Xmn I یێ نەوەك هەڤ (+/−) هاتە دیتن لدەف 32.7 و 21.8٪ لدویف ئێك. بلندترین ئاستێ هیموگلوبینێ F هاتە دیتن لدەف ئەوێن (+/+) و کێمترین هاتە دیتن لدەف ئەوێن (−/−) و ئەف پێزانینە یا گرنگ بوو (p= 0.029).

دەرئەنجام: مشەشێوەییا Xmn I گهورکەرەکێ زکماکی یێ گرنگە بو دژواریا تالاسیمیا بێتا لدەف کوردێن عیراقی و گرێدان یا هەی دگەل بلندبوونا هیموگلوبینێ F. پتر ڤەکولین لسەر گهورکەرێن زکماکی پێدڤی نە بو دەستنیشانکرنا بەشداربوونا وان د جورەیێنن شێوەیی یێن ڤێ نەخوشیا زکماکی یا گرنگ.

الخلاصة

تعدد أشكال Xmn I في مرضى بيتا تالاسيميا في دهوك

خلفية واهداف البحث: يوجد العديد من المعدّلات الوراثية المؤثرة في الاشكال المظهرية لمتلازمة فقر دم البحر المتوسط ومن المعدلات المهمة في شرقي البحر المتوسط هو polymorphism XmnI و لكن لاتوجد دراسة بخصوص المساهمة النسبية لهذا المعدل للمرضى العراقيين.

طرق البحث: تم دراسة مجموعه من 107 مرضى فقر دم البحر الابيض المتوسط (52 ثالاسيميا وسطية و 55 ثالاسيميا كبرى) . وقد تم مراجعة ملفاتهم المرضية ثم عمل صورة دم كاملة و تحديد نسب A2 و F بطريقة HPLC و استخلاص الدنا . ومن ثم استعمل الدنا لتحديد وجود المعدل XmnI بطريقةPCR–RFLP.

النتائج: وجد ان المعدل Xmn1متماثل الزيجة (+/+) وجد في 19.2 % و1.8 % من مرضى الثالاسيميا الوسطية و الكبرى على التوالي (p =0.0028). أما متباين الزيجة XmnI (− /+) فقد وجدت في 32.7% و 21.8 % على التوالي. وقد وجد انه ومن بين 107 مريضا مشمولا بالدراسة فأن الأنماط الوراثية (+/+)، (+/−) و(−/−) بالتتابع إرتبطت بالأحتياج لنقل دم سنوي وسيط 0, 3.5 و11 على التوالي مع احتياج اقل للمرضى الحاملين للانماط +/+ و+/− مقارنة مع غير الحاملين (0.02 و (p =0.004 على التوالي. وقد وجد ان اعلى نسبة Hb F كانت موجودة في الاشخاص حاملي النمط (+/+) وأقلّ نسبة في الاشخاص حاملي نمط (−/−) و بمستوى معنوي مهم (p =0.029).

الاستنتاجات: أن النمط الوراثي لـ Xmn I هومعدل وراثي مهم لتحديد شدة فقر البحر الابيض المتوسط بين الأكراد العراقيين، و ان وجوده مرتبط بارتفاع مستوى Hb F عند المقارنة مع غير الحاملين له . ويبدو ان هناك معدلات وراثية أخرى تحتاج للدراسة لتحديد دورها في المساهمة في كشف الاختلافات المظهرية في هذا المرضى الوراثي المهم.

INCIDENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF PSEUDOMONAS AERUGINOSA IN BURNS INFECTIONS IN DUHOK CITY

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ABSTRACT

Background and objectives *Pseudomonas aeruginosa* is an opportunistic pathogen causing severe, acute and chronic nosocomial infections. The organism is generally resistant to numerous antimicrobial agents, making the treatment of infections further difficult. The aim of this study was to determine the incidence of *Pseudomonas aeruginosa* involved in burns infections. Also, the susceptibility and resistotyping patterns of the isolates to commonly prescribed antibiotics were studied.

Methods During a period of six months between July and December, 2010, a total of 159 samples from burns infections, using sterile cotton swabs, were collected from Burns Hospital in Duhok city. The samples were plated on Blood agar and MacConkey agar and the isolates were identified by routine procedures. Antibiotics susceptibility and resistant profiles to 14 commonly prescribed antibiotics were performed by the disc diffusion method using Mueller-Hinton agar.

Results Out of the 159 samples collected from burns infections, 116 samples were showed bacterial growth, 76 (47.7%) were *Pseudomonas aeruginosa*, followed by *Klebsiella pneumoniae* 20 (12.5%), *Escherichia coli* 10 (6.2%), *Staphylococcus aureu*s 8 (5 %), *Staphylococcus epidermidis* 2 (1.2%), and no growth 43 (27 %). The results showed that the occurrence of *Pseudomonas aeruginosa* was higher than the other groups of bacteria. The sensitivity pattern of *Pseudomonas aeruginosa* revealed that the organism was highly sensitive to impenem (98.6%) followed by piperacillin (60.5), ciprofloxacin (57.8), and amikacin (48.6%). On other hand, chloramphenicol (19.7%), doxycycline (10.8%), ceftazidine (10.8%), erythromycin (6.5%), gentamicin (3.9%), cefotaxime (3.9%), amoxiclav (3.9%), tetracycline (3.9%), vancomycin (3.9%) and cefixime (2.6%) showing the lowest percentages sensitivity. Resistant profiles were determined. A total of 12 different resistotype patterns were obtained; common resistotype were 5 and 11.

Conclusions This study shows that there is an increased rate of incidence of *Pseudomonas aeruginosa* in burns infections and most of these isolates were multi-drug resistant and showed different resistotyping patterns.

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Key words: *Pseudomonas aeruginosa*, Multidrug-resistance, Burns infection, Resistotyping patterns, Antibiotics

Pseudomonas aeruginosa is a gram-negative rod measuring 0.5 - 0.8 µm by 1.5 to 3.0 µm, commonly found in soil and water.[1] *Pseudomonas aeruginosa* can

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cause infections virtually anywhere in the body, but urinary tract infections, pneumonia (especially in cyst fibrosis patients) and wound infections (especially burns) predominate. From these sites, the organism can enter the blood, causing sepsis. *Pseudomonas aeruginosa* have a remarkable ability to withstand disinfectants, this accounts in part for their role in hospital-acquired infections, they have been found growing in hexachlorophene-containing soap solutions, in antiseptic and in detergents.[2] Almost all the clinical cases of *Pseudomonas aeruginosa* infection can be associated with the compromise of host defense such as burn patients. While many cases of *Pseudomonas aeruginosa* infection can be attributed to general immuno-suppression e.g. AIDS patients.[3,4]

Most pseudomonad burns infections are established through colonization of the burns wound by the patient's own flora or from the environment. Most fatalities (usually arising from septicemia) are associated with full-thickness burns, and there is a strong correlation with the percentage area of the burn.[5] Patients with burns infected with *Pseudomonas aeruginosa* have an increased mortality rate and longer hospital care compared to non-infected patients. They also have an increased number of surgical procedures and higher associated antibiotic costs.[6] Burn hospitals often harbor multidrug-resistant *Pseudomonas aeruginosa* that can serve as the source of infection. *Pseudomonas aeruginosa* has been found to contaminate the floors, bed rails, and sinks of hospitals, and has also been cultured from the hands of nurses.[7]

Concerning multi-drug resistance, Hsueh et al 1998, reported multi-drug-resistant strain of *Pseudomonas aeruginosa* over a period of several years was carried by some patients asymptomatically through several rounds of antibiotic treatment for *Pseudomonas aeruginosa* infections.[8] This scenario can be worse during the spread of *Pseudomonas aeruginosa* from one patient to another; the persistence of this strain takes place in patients throughout several courses of antibiotic treatment.[5] It has been proved that during admission of patients in burn centers, a limited number of common strains cross-contaminate burn victims mostly when their lesions scrubbed in the bathroom.[9]

Pseudomonas aeruginosa exhibits resistance to a variety of antimicrobials including beta lactams. Carbapenems are often used as antibiotics for treatment of infections caused by beta lactam resistant *Pseudomonas aeruginosa*.[10] The aim of the present study was to find out the incidence and antibiotic susceptibility of *Pseudomonas aeruginosa* isolates recovered from the burns infections, also establishment the resistotype patterns of these isolates.

METHODS

The specimens were collected from patients aseptically with sterile cotton wool swab suffering from burns infections at burns hospital in Duhok city from July and December 2010. Swabs being routinely processed by the Department of Laboratory Service at Burns Hospital. Several media and tests were used for the isolation; identification and testing the susceptibility of the isolates for commonly used antibiotics. The media used are:

Blood agar (with 5-7% defibrinized blood), MacConkey agar, chocolate agar, nutrient agar, Mannitol salt agar. Simmons citrate agar, kligler Iron Agar (KIA), Mueller-Hinton agar, Sulfide formation indole production, motility Test (SIM), Nutrient agar, Methyl Red-Voges Proskauer broth, Thioglycollate broth, Coagulase, Catalase, Urease, Oxidase tests were used for the identification. All of the above media and reagents were obtained from (Difco. USA).

The media were prepared according to manufacturers instructions in 500 mL bottle and sterilized by autoclaving at

121°C for 20 minutes. All wound swabs collected for bacteriological investigations during the period of this study were treated according to established method of treating wound swabs.[11] Gram stain preparations were made from all the swabs the plates were incubated at 37°C for 18-24 hourrs in an incubator. The plates were read the following day but extended to 48 hourrs if there was no bacterial growth within 24 hours. Isolated colonies were subjected to Gram staining technique and biochemical tests for identification.

Antibiotic sensitivity tests were carried out on isolated and identified colonies of *Pseudomonas aeruginosa* using commercially prepared antibiotic sensitivity disc using Kirby-Bauer method.[12].

RESULTS

A total of 159 samples were collected from burns hospital, all specimens were directly transferred to the microbiology laboratory and cultured to the appropriate media (as described in methods).

Table 1 shows the most causative agents of burns infections were *Pseudomonas aeruginosa* 76 isolates (47.7%), followed by *Klebsiella pneumonia*e 20 isolates (12.5%). The lowest causative agents of burns infections were *Escherichia coli, Staphylococcus aureus*, and *Staphylococcus epidermidis.*

Table 1. Frequency and percentage of microorganisms isolated from patients in burns infections

Microorganism	Number of isolation (%)
Pseudomonas aeruginosa	**76 (47.7)**
Klebsiella pneumoniae	**20 (12.5)**
Escherichia coli	**10 (6.2)**
Staphylococcus aureus	**8 (5.0)**
Staphylococcus epidermidis	**2 (1.2)**
No growth	**43 (27.0)**
Total	**159 (100)**

Table 2 shows sensitivity patterns of *Pseudomonas aeruginosa* isolated from patients with burns infections. The organism was sensitive to imipenem followed by piperacillin, ciprofloxacin, and amikacin.

Table 3 shows the resistotyping patterns of *Pseudomonas aeruginosa* isolates to commonly used antibiotics. It was found that all of the isolates were multiple resistant, i.e. resistant to more than one antibiotic. The obtained results of resistotyping patterns (resistant profiles) revealed that 58 isolates were multiple drug resistant to antibiotics and 12 different patterns were found.

Table 2. Susceptibility test of Pseudomonas aeruginosa isolated from patients in burns infections

Antibiotics	Symbol	Disc potency (µg)	Susceptibility (%)
Imipenem	**Imp**	**10**	**98.6**
Piperacilin	**PRL**	**100**	**60.5**
Ciprofloxacin	**CIP**	**5**	**57.8**
Amikacin	**AK**	**30**	**48.6**
Chloramphenicol	**C**	**10**	**19.7**
Ceftazidime	**CAZ**	**30**	**10.8**
Doxycycline	**DOX**	**30**	**10.8**
Erythromycin	**E**	**15**	**6.5**
Gentamicin	**CN**	**10**	**3.9**
Cefotaxime	**CTX**	**30**	**3.9**
Vancomycine	**VA**	**30**	**3.9**
Amoxiclav	**AMV**	**5**	**3.9**
Tetracycline	**TE**	**30**	**3.9**
Cefixime	**CFM**	**30**	**2.6**

Table 3. Resistotyping patterns of Pseudomonas aeruginosa isolates

Resistotyping patterns	Resistance Spectrum Phenotype	No. of isolates (%)
Resistotype 1	DOX, CTX, CAZ, TE, CFM, VA, E, CN, AMC	2 (3.44)
Resistotype 2	AK, CTX, CAZ, TE, CFM, VA, E, CN, AMC	3(5.1)
Resistotype 3	CIP, DOX, CTX, TE, CFM, VA, E, CN, AMC, C	4(6.8)
Resistotype 4	AK,PRL, CTX, CAZ, TE, CFM, VA, E, CN, AMC	2(3.4)
Resistotype 5	DOX, CTX, CAZ, TE, CFM, VA, E, CN, AMC,C	9(15.9)
Resistotype 6	PRL, DOX, CTX, CAZ, TE, CFM, VA, E, CN, AMC	4(6.9)
Resistotype 7	AK, DOX, CTX, CAZ, TE, CFM, VA, E, CN, AMC	3(5.1)
Resistotype 8	CIP, DOX, CTX, CAZ, TE, CFM, VA, E, CN, AMC, C	5(8.2)
Resistotype 9	AK, CIP, DOX, CTX, CAZ, TE, CFM, VA, E, CN, AMC	7(12)
Resistotype 10	PRL, CIP, DOX, CTX, CAZ, TE, CFM, VA, E, CN, AMC	5(8.2)
Resistotype 11	AK, PRL, DOX, CTX, CAZ, TE, CFM, VA, E, CN, AMC	9(15.9)
Resistotype 12	AK, PRL, CIP,DOX, CTX, CAZ, TE, CFM, VA, E, CN, AMC	5(8.2)
Total		58(100)

DISCUSSION

The burn wound is considered one of major health problem in the world, and infection is one of the frequent and severe complications in patients who sustained burns.[13,14] The burn wound represents a susceptible site of opportunistic colonization by organisms of endogenous and exogenous origin. Patient factors such as age, extent of injury and depth of burns in combination with microbial factors determine the likelihood of invasive burn wound infection.[15]

In our study *Pseudomonas aeruginosa* was found in burns infections 76 (47.7%), be the most common organism isolated during 6 months from burns hospital, followed by *Klebsiella pneumonia*e (12.5.6%). This is in agreement to other studies which showed *Pseudomonas* as the most common infective organism in burns patients. Naser et al 2003, from Cairo, Egypt has reported *Pseudomonas aerugionas* was the most frequent isolate (21.6%), followed by *Klebsiella pneumoniae* (15.2%) and *Staphylococcus* 11.6%.[16] Taneja et al 2004, from Chandigarh, India have reported *Pseudomonas aerugionas* as the most frequent isolates (54.2%) followed by *Staphylococcus aureus* (20.8%). Other study in india indicated that the *Pseudomonas aeruginosa* was predominant in burns infections.[17]

Nowadays, the prevalence of *Pseudomonas aeruginosa* and the new resistant strains continue in both community-acquired pathogens and hospital originated infections.[18] In our study most of *Pseudomonas aerugionas* isolates were highly sensitive to imipenem (98.6%) followed by piperacillin (60.5%), ciprofloxacin (57.8%), and amikacin (48.6%). In a study conducted by Revathi et al, *Pseudomonas aerugionas* was most susceptible to ceftizidime (83%) and cefoperazone (82%).[19] In a study carried out in Turkey by Inan et al, isolated 68% of *Pseudomonas aeruginosa* strains and 60-83% of the antibiotics resistant strains were from ICU patients. In the same study, resistance was detected against ceftazidime 34%, imipenem 26%, gentamicin 67%, and amikacin 26%.[20]

Our study showed that the susceptibility rate of *Pseudomonas aeruginosa* to gentamicin was very low as 3.9%. Reports of the susceptibility of *Pseudomonas aeruginosa* to gentamicin have ranged from as low as 49.8% in Greece, to 70% in Turkey, to as high as 96.6%, in the United Kingdom.[21] In Trinidad and Tobago, 80 and 78.4% of isolates were susceptible to ceftazidime and gentamicin respectively.[22] Report from France have shown *Pseudomonas aeruginosa* susceptibility rates of 78.5 and 61.7% to ceftazidime and ciprofloxacin, respectively.[23] Increased resistance was

observed in Russia where only 25% of isolates were susceptible to gentamicin.[24] While in Bangladesh 49 and 79% of isolates were susceptible to tobramycin and ciprofloxacin respectively.[25]

Consistent with these findings, resistance to gentamicin of *Pseudomonas aeruginosa* is increasing progressively in our country. The most important risk factors are obvious, such as excessive consumption of antibiotics exerting selective pressure on bacteria, the frequent use of invasive devices and relative density of a susceptible patient population in burns units.[26]

Ciprofloxacin resistance rate was 42.2% in our study, 27.4% in Turkey,[20] 32% in Spain,[27] 31.9% in Italy,[28] 26.8% in Latin America,[29] 31% in India,[17] and 100% in Iran.[30] Thus, in burns infections, empirical antibiotic treatments should be avoided and treatment should be carried out using antibiotic susceptibility tests. While piperacillin resistance rate was 39.5% in our findings, 10% in Spain,[27] 12% in Italy,[28] 14% in Latin America,[29] 28.7% in Turkey,[20] 20% in India,[17] and 100% in Iran.[30] Most studies conducted in the world shows that most common drugs are resistant to the organism isolated like ampicillin, erythromycin and cefotaxime.[31,32] These findings are consistent with our findings.

Resistotyping is a phenotypic method that consists of testing bacterial strains against a set of arbitrarily chosen antibiotics, whereby, a resistance pattern that is characteristic of a strain is generated and, is believed to describe the isolates for epidemiological purposes. Obtained results of this study revealed that the 58 isolates of *Pseudomonas aeruginosa* from burns infections were belonged to 12 distinct resistotype patterns. Resistotype 5 and 11 have much higher frequency rate comprising 15.9% (for each one) of the isolates. Studies in Spanish,[33] Brazil,[34] Iran,[35] and Iraq,[36] conducted resistant profiles of strains *Pseudomonas aeruginosa*. Moreover, those isolates, from various patients, were identical on the basis of disk susceptibility patterns, indicating relatedness among them. In general, this simple typing system provide discriminatory between strains and able to determine relatedness among isolates of *Pseudomonas aeruginosa* in order to tracing the source of infections in our environment.

This study concluded that there is an increased rate of incidence of *Pseudomonas aeruginosa* in burns wounds infections followed by *Klebsiella pneumoniae, Escherichia coli*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. This is in agreement with survey studies carried out in various hospitals. The infection appears to be common in burns hospitals owing to the burns units are a very susceptible habitat for bacterial colonization and excessive use of empiric antibiotic treatment. The reason for this increase in burns infections rate with prolonged hospitalization is primarily due to colonization of patients with hospital-acquired resistant microorganisms. Resistance of *Pseudomonas aeruginosa* to antibiotics is very definitely associated with overuse of broad-spectrum antibiotics in hospitals, lead to different resistotyping patterns of antibiotics have been determined. Therefore, new and more effective antibiotics may be needed.

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پوختە

بەلاڤبوون وهندك جورى دژە زیندە هى بەکتریاى Pseudomonas aeruginosa ژ نەخوشیت سوتنى ل نەخوخشخانا سوتنا ل باریّزگەها دهوکى

پێشەکى و ئارمانج: ئە ڤ بە کتریایە Pseudomonas aeruginosa دهیتە هژمارتن کو دبیتە هویئ توش بونا نە خوشیئت دریژخایە ین وئیشین دژوار ژوان نە خوشییت بە رگریا لە شى کیم لدە ڤ وان، ونە خوشییت سوتنى، و ئە ڤ بە کتریایە بە رگریى ل خودکە ت بشیویە کى سە رە کى ژ هندە ك دژە زیندە هى antibiotic کو ب زە حمە ت بهیتە چارە سە رکرن بو وان نە خوشین توشى ڤى جورە بە کتریایى بن.ئارمانج ژ ڤى خاندنى ئە وە کو هە بونا ڤى جورئ بە کتریایى ژ نە خوشین سوتنى یاهە ى، ماوە یە کى هە ستیار وهیلا بە رگریى بوڤان جورا وهندە ك دژە زیندە هى کودهیتە هژمارتن وە ك چارە سە رى بو ڤان نە خوشییت توژى ڤى جورە بە کتریایى دبن.

ریکێن ئەکولینێ: د ڤى ڤە کولینى دا دیاربى 159 سامبل هاتنە کوم کرن یان دیارکرن دماوى تە موز –تشرینى یە کە م 2010 ژنە خوشیت سوتنى ل نە خوشخانا سوتنا ل باریزگە ها دهوکى. و ئە ڤ سامبلە هاتنە کوم کرن بە ریکا بارچە بە مبیى یە کى باقژ ژهندە ك جهیت برینیت سوتنى دبە ن ژ نە خوشییت سوتنى دى جینین ل هندە ك شوینیت چاندنى ود کە ینە حاچنى دا ماوى 24–48 دە مژمیرا ل تاقیگە ها نە خوشخانا سوتنى، و پشتى ڤى چە ندى دى ڤان جورى سامبلا بە ش بە ش کە ین بشیویە کى هە رە مە کى. پشتى هنگى دى پشکنینا هە ستیار زیندە کى، و ئینانا جوریت بە رگریى ب کار ئینین disk diffusion.

ئەنجام: هژمارا سامبلە 116،159 سامبلە بە کتریا دیاربین، دناڤ برا وان 76 (47.7٪) Pseudomonas aeruginosa، 20 (12.5%) Klebsiella pneumoniae، 10 (6.2%) Escherichia coli، 8 (5%) Staphylococcus aureus، 2 (1.2%) Staphylococcus epideremidis و 27 (43٪) چ ئە نجاما نادە تە مە. ئە نجام هاتنە دیارکرن کو ریژا Pseudomonas aeruginosa پتربى ژ هە مى جوریت دى ین بکتریا و ریژە کا کیم Escherichia coli، Staphylococcus aureusو Staphylococcus epideremidis ژ نە خوشین توشى ڤان بە کتریایا بین. پشکنینا هە ستیار ژ جورى Pseudomonas aeruginosa دهیتە وە رگرتن ژسوتین توشى ڤى بە کتریایى دبن هە ستیارى پتر imipenem، ciprofloxacin، piperacillin، amikacin ژجهە کى دى دیاربى ل دژە زیندە هى دا کو ریژا هە ستیاریى کیمە ceftaxime، tetracycline، amoxiclav، gentamicin، cefotaximeو vancomycin د ڤى جورە بە کیریایى دا. وهاتە دیارکرن ل پشکنیندا جورى بە رگریى 12 بە رگریا جیاواز وجورى بە ربە لا ڤ بە رگریا 5 و 11.

دەرئەنجام: : دیاربى دخاندنى دا کوریژە یە کا زیدە Pseudomonas aeruginosa یاهە ى ژنە خوشیت سوتنى یت کو توشى ڤى جورە بە کتریایى بین و پترتریا ڤان جورە بە کتریا دیاردبن کو بە رگریى ژخودکە ن ژ دژە زیندە هییا ودیاربى جورین بە رگرین جیاواز.

الخلاصة

نسبة تواجد وأنماط المقاومةالمضادادت الحياتية لبكتريا الزوائف الزنجارية المعزولة من أصابات الحروق في مستشفى الحروق في مدينة دهوك

خلفية واهداف البحث: تعتبر Pseudomonas aeruginosa بكتريا انتهازية تسبب إصابات مرضية حادة ومزمنة في مرضى ذات المناعة القليلة ومرضى القسطرة وكذلك مرضى الحروق. هذه البكتريا بصورة رئيسية مقاومة لعدد كبير من المضادات الحياتية، مما يصعب علاج الحالات المصابة بهذه البكتريا.الهدف من هذه الدِراسة هو لايجاد نسبة تواجد بكتريا Pseudomonas aeruginosa في اصابات التهابات الحروق، وتحديد مدى حساسية هذه العزلات لمعظم المضادات الحياتية المتداولة، وكذلك اظهار انماط المقاومة لهذه العزلات.

طرق البحث: تضمنت هذه الدراسة 159 عينة جُمعت من الاصابات البكتيرية لالتهابات الحروق في مستشفى الحروق/مدينة دهوك، في الفترة الزمنية بين تموز – كانون الاول 2010. وتم جمع العينات بإستخدام مسحات قطنية معقمة وأخذ عينات من القيح من إصابات الحروق وزرعها في الاوساط الزرعية التشخيصية والتحضين لمدة 24–48 ساعة في قسم المختبرات في مستشفى الحروق، وتم تشخيص العُزلات باجراء الاختبارات التشخيصية الروتينية.تمّ إخضاع العُزلات إلى إختبار فحص الحساسية للمضادات الحياتية بإستخدام طريقة إنتشار القرص، وايضاً إيجاد الانماط المقاومة للعُزلات.

النتائج: العدد الكلي للعينات 159 عينة، 116 عينة اظهرت زرع بكتيري، مِن ضمنها 76 (47.7%) عُزلة Pseudomonas aeruginosa و 20 (12.5%) عُزلة Klebsiella pneumoniae، 10(6.2%) Escherichia coli، 8(5%) Staphylococcus aureus، 2 (1.2%) Staphylococcus epidermidis و 43 (27%) لم يعطي زرعاً بكتيرياً. أوضحت النتائج أن نسبة Pseudomonas aeruginosa كان الاكثر من بين المجاميع البكتيرية في اصابات الحروق، والنسبة الاقل للاصابات البكتيرية في الحروق كانت Escherichia coli، Staphylococcus aureus و Staphylococcus epidermidis. أُجري إختبار الحساسية لِعُزلات Pseudomonas aeruginosa ألمأخوذة مِن الاصابات البكتيرية لالتهابات الحروق حيث كانت اكثر حساسية للمضادات الحياتية imipenem تليها piperacillin، ciprofloxacin وamikacin بينما اظهرت اقل نسبة حساسية للمضادات الحياتية ceftaxime تليها tetracycline، gentamicin، cefotaxime، amoxiclav و vancomycin. كذلك تمّ إجراء تحليل انماط المُقاومة للمضادات الحياتية حيث ظهرت 12 نمط مقاوم مختلف، وانّ الانماط السائدة كانت نمط المقاومة 5 و 11.

الاستنتاجات: اظهرت هذه الدراسة زيادة في نسبة وجود Pseudomonas aeruginosa في الاصابات البكتيرية لالتهابات مرضى الحروق واكثر هذه العزلات اظهرت مقاومة متعددة للمضادات الحياتية وكذلك اظهرت انواع مختلفة من نمط المقاومة.

BIOFILM FORMATION BY METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) WITHIN HOSPITAL AND COMMUNITY ACQUIRED URINARY TRACT INFECTIONS

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ABSTRACT

Background and objectives Methicillin resistant *Staphylococcus aureus* is a significant cause of life-threatening human infections, which can switch from planktonic forms (i.e. single cells) to biofilms. Biofilm formation was often-lower susceptibility to antibiotic treatments and development of chronic infections. The study was investigated biofilm formation by methicillin resistant *Staphylococcus aureus* isolated from patients with urinary tract infection. Meanwhile assess the relationship between biofilm formation and antibiotic resistance.

Methods *Staphylococcus aureus* were isolated and identification by standard methods from urinary tract infections at three teaching hospitals in Erbil city. Methicillin resistant *Staphylococcus aureus* were detected by PBP2a. Heterogeneity of of methicillin resistant *Staphylococcus aureus* was determined by efficiency of plating method. Minimum inhibitory concentration of antibiotics was determined by agar dilution method. Biofilm forming ability of methicillin resistant *Staphylococcus aureus* was investigated.

Results Methicillin resistant *Staphylococcus aureus* were resistances to 10.92±3.17 antibiotics. The percentage of biofilm formation by methicillin resistant *Staphylococcus aureus* was 82%. Strong biofilm formations were resistance to13.40±2.51 antibiotics, which is statistically higher than biofilm negative (Mean±SD = 6.56±1.51).

Conclusions Most methicillin resistant *Staphylococcus aureus* were biofilm forming. Biofilm formation was correlated with multiple antibiotics resistance and to heterogeneous of methicillin resistant *Staphylococcus aureus*.

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Key words: Biofilm, MRSA, Staphylococcus aureus, Antibiotics, UTI, Infection

Biofilm is a multicellular creature made up of bacteria that irreversibly attached to a surface or interface, embedded in a matrix of extracellular polymeric substances.[1,2] Biofilm formation is a complex developmental process involving attachment and immobilization on a surface, cell-to-cell interaction, microcolony formation, formation of a confluent biofilm, and development of a three dimensional biofilm structure.[3,4] Biofilms are responsible for several chronic diseases and show much greater resistance to antibiotics than their free-living counterparts.[5] Bacterial cells within biofilms are inherently resistant to antimicrobial treatment and are difficult to eradicate from the infected individual. The high rates of morbidity and mortality associated with these infections.[6] Biofilms are very hard to eradicate and responsible for a significant number of nosocomial and

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indwelling device-associated infections.[7-9] Biofilms are notoriously difficult to eradicate and are a source of many chronic infections. According to the National Institutes of Health, biofilms are medically important, accounting for over 80% of microbial infections in the body.[4]

For decades, *Staphylococcus aureus* has been recognized as an important cause of disease around the world. It has become a major pathogen causing hospital and community acquired infections.[10,11] *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* (MRSA) are known to form biofilms on a variety of materials.[12] They can persist in clinical settings and gain increased resistance to antimicrobial agents through biofilm formation, which appears to be a bacterial survival strategy. Therefore, biofilms formed by MRSA have become resistant to the majority of antimicrobial agents. Due to gained multi-resistance, infections caused by MRSA are very difficult to treat.[10] Factors contributing to the occurrence of MRSA infections are cross transmission via the hands of healthcare workers and high selective pressure exerted by broad-spectrum antibiotic therapy,[11] which is becoming increasingly difficult to treat given the ever-increasing incidence of MRSA and, more recently, the emergence of glycopeptide resistance.[13]

MRSA has emerged as a major clinical and epidemiological problem in hospitals. A distinctive feature of MRSA strains is their resistance not only to all β-lactam antibiotics, but also to a wide range of other antimicrobials, which makes MRSA infections difficult to manage and costly to treat.[14] In recent years, the increasing incidence of urinary tract infections (UTI) caused by *Staphylococcus aureus* has been noted at the urology ward. The incidence of MRSA induced UTI is getting higher these days, especially for inpatients with an indwelling urinary catheter or those who are immunocompromised.[10,15]

METHODS

Midstream urine samples were aseptically collected then cultured on blood agar (Oxoid, England) and mannitol salt agar (Oxoid, England) by standard techniques as soon as possible[16,17] from 1367 patients with UTIs had documented pyuria (WBC>5/hpf) at Maternity, Rizgary and Hawler teaching hospitals in Erbil city from June 2010 through April 2011. Data information's were collected from patients, which included age, sex, and urinary catheterization. The infections were classified into community or hospital acquired infections based on Centers for Disease Control and Prevention definitions.[18] MRSA infections in which MRSA was recognized in patients after 72 hours of hospitalization were considered as hospital acquired MRSA, this is the usual accepted duration of hospitalization required to develop the hospital-acquired infections. MRSA infections in outpatients were considered as community acquired MRSA. Exclusion criteria was pregnant woman, age less than 18 years, patient's hospitalizations less than 72 hours and outpatients have been hospitalizations in the last 21 days.

Staphylococcus aureus was identified by colony morphology, Gram staining, fermentation of mannitol, tube coagulase test, and Avipath Staph (Omega, UK).[19] MRSA was detected using PBP2a kit (Oxoid, Japan) for detection PBP2a on cell wall of MRSA, which performed by sufficient colonies of *Staphylococcus aureus* suspended in 200µl extraction reagent 1 and heated in boiling water for 3 minutes. Tubes were cooled and 50µl extraction reagent 2 was added. Tubes were centrifuged at 1500xg for five minutes. Fifty microliters of suspension was mixed with 50µl sensitized latex suspension and rotated manually for 3 minutes while looking for agglutination, i.e., MRSA positive.[20,21] Heterogeneous of MRSA was determined quantitatively by efficiency of plating method.[22]

Overnight cultures of MRSA isolates were grown at 37°C in brain heart infusion broth (HiMedia, India) supplemented with 2% glucose and 2% sucrose. The culture was diluted 1:100 in brain heart infusion, and MRSA suspensions (200µL) were transferred to individual wells of a flat-bottom 96-well microplates (Costar, USA). After 48 hours at 37°C without shaking, wells were gently washed three times with distilled water, dried in an inverted position, and stained with 300µL of 2% crystal violet solution in water for 45 min. After staining, plates were washed 3 times with distilled water, and destained with 200µL of ethanol/acetone (95:5, vol/vol). A total of 200µL from each well was transferred to a new microplates, and analysis at optical density (OD) of 570 nm. Each assay was performed in triplicate, and the mean OD_{570} value of tested wells was applied to biofilm forming ability. As a control, uninoculated medium was used to determine background OD. The mean OD_{570} value from the control wells was subtracted from the mean OD_{570} value of tested wells. The biofilm formation was divided into strong (OD_{570} ≥0.5), medium (OD_{570} ≥0.2 to <0.5), weak (OD_{570} 0 to < 0.2), and negative biofilm formation.[23,24]

The minimum inhibitory concentration (MIC) was determined by the agar dilution method according to EUCAST (2001),[25] and BSAC (2010)[26] guidelines for Penicillin G (Sigma-Aldrich), Cefotaxime (Sigma-Aldrich), Ceftriaxone (Mepha), Cefepime (Exir), Tetracycline (Sigma-Aldrich), Doxycycline (Sigma-Aldrich), Amikacin (Sigma-Aldrich), Tobramycin (Sigma-Aldrich), Erythromycin (Sigma-Aldrich), Azithromycin (Fluka), Clarithromycin (Sigma-Aldrich), Ciprofloxacin (Fluka), Gatifloxacin (Cipla), Levofloxacin (Sigma-Aldrich), Moxifloxacin (Bayer), Ofloxacin (Sigma-Aldrich), Clindamycin (Sigma-Aldrich), Rifampicin (Sigma-Aldrich), and Chloramphenicol (Sigma-Aldrich). The bacteria were classified as susceptible or resistant according to BSAC breakpoint criteria.[26]

All statistical analyses were performed by Statistical Package for Social Sciences (SPSS). Descriptive statistics were given as arithmetic mean ± SD (standard deviation) and t-test. Comparisons between different groups were evaluated by one-way ANOVA with Duncan test at $p<0.05$. Correlation analyses were used to assess the relationship between two variables. All results were considered statically significant at the $p< 0.05$ level.

RESULTS

Overall 157 (11.49%) *Staphylococcus aureus* isolates from 1367 UTIs were isolated; the prevalence of MRSA was 31.85% (50 of 157 *Staphylococcus aureus*) that isolated from 27 (54%) females and 23 (46%) males. The mean age of females (52.44±10.65) was statistically older than males (46.30±10.55) (Table 1). Statistical comparison was done by using Duncan test at $P<0.05$ for multiple comparison of 20 antibiotics revealed that the lower mean of MIC±SD were 0.09±0.06, 0.83±0.58, 0.96±0.14 and 0.96±0.57 µg/ml for Rifampin, Clarithromycin, Gatifloxacin and Clindamycin, respectively. However, significant differences were observed between MIC hospital and community acquired MRSA for Ceftriaxone, Amikacin, Azithromycin, Gatifloxacin and Clindamycin (Table 2).

Table 1. Gender and age of UTI patients with MRSA

Gender	No. (%)	Age (years)		
		Mean±SD	Min	Max
Female	27 (54)	52.44±10.65	24	64
Male	23 (46)	46.30±10.55	23	68
Total	50 (100)	49.62±10.94	23	68

Significant (t-value = 2.04, p-value = 0.047).
Min: Minimum; Max: Maximum

Table 2. MIC and antibiotics resistances of 34 hospital and 16 community acquired MRSA

Antibiotic	Antibiotic MIC					Antibiotic resistance		
	Mean±SD of MIC (mg/L)		Statistical analysis		Total Mean±SD (mg/L)	Hospital acquired	Community acquired	Total
	Hospital acquired	Community acquired	t-value	p-value		No. (%)	No. (%)	No.(%)
Penicillin G	70.59±25.86	58.00±31.39	1.50	0.14	66.56±28.06^{h}	34(100)	16(100)	50(100)
Cefotaxime	56.59±20.60	60.13±28.04	0.50	0.62	57.72±23.01^{g}	30(88.24)	15(93.75)	45(90)
Ceftriaxone	107.35±39.92	72.00±29.79	3.15	<0.01	96.04±40.28^{i}	32(94.12)	16(100)	48(96)
Cefepime	13.53±4.70	14.50±7.85	0.55	0.59	13.84±5.83^{e}	32(94.12)	16(100)	48(96)
Tetracycline	10.65±7.09	10.38±9.51	0.11	0.91	10.56±7.84^{de}	23(67.65)	9(56.25)	32(64)
Doxycycline	3.82±3.55	4.47±4.52	0.55	0.59	4.03±3.85^{abc}	14(41.18)	9(56.25)	23(46)
Gentamicin	10.71±6.74	9.41±7.74	0.61	0.55	10.29±7.02^{de}	28(82.35)	11(68.75)	39(78)
Amikacin	24.82±12.20	16.50±14.21	2.13	0.04	22.16±13.32^{f}	25(73.53)	7(43.75)	32(64)
Tobramycin	1.97±2.55	1.50±1.91	0.66	0.52	1.82±2.35^{ab}	5(14.71)	2(12.50)	7(14)
Erythromycin	6.43±4.17	9.53±21.29	0.58	0.57	7.42±12.35^{bcd}	29(85.29)	11(68.75)	40(80)
Azithromycin	12.38±6.17	6.69±8.81	2.65	0.01	10.56±7.52^{e}	29(85.29)	9(56.25)	38(76)
Clarithromycin	0.79±0.29	0.91±0.94	0.46	0.65	0.83±0.58^{a}	22(64.71)	7(43.75)	29(58)
Ciprofloxacin	5.85±5.66	5.38±10.54	0.21	0.84	5.70±7.46^{abcd}	25(73.53)	4(25.00)	29(58)
Gatifloxacin	0.94±0.16	1.00±0.00	2.10	0.04	0.96±0.14^{a}	0(0.00)	0(0.00)	0(0.00)
Levofloxacin	2.24±1.50	2.25±2.29	0.03	0.98	2.24±1.77^{ab}	2(5.88)	2(12.50)	4(8)
Moxifloxacin	1.21±0.76	1.56±2.52	0.55	0.59	1.32±1.54^{a}	4(11.76)	2(12.50)	6(12)
Ofloxacin	3.29±2.05	2.94±2.38	0.54	0.59	3.18±2.14^{ab}	16(47.06)	7(43.75)	23(46)
Clindamycin	1.09±0.60	0.69±0.40	2.79	0.01	0.96±0.57^{a}	22(64.71)	4(25.00)	26(52)
Rifampicin	0.10±0.06	0.08±0.06	0.85	0.40	0.09±0.06^{a}	14(41.18)	2(12.50)	16(32)
Chloramphenicol	10.35±4.42	8.25±4.95	1.51	0.14	9.68±4.65^{cde}	12(35.29)	4(25.00)	16(32)

The same letters mean no significant difference, the different letter mean significant difference at $p<0.05$.

Numbers of antibiotics resistance (Mean±SD) of MRSA were 10.92±3.17, which is higher in hospital acquired infection (11.59±2.69) than community acquired infection (9.50±3.71) (Table 3). The percentage of urinary catheterized of patients with MRSA infection were 66% that resistance to 11.79±2.46 antibiotics, which is statistically higher than non-catheterized patients (Mean±SD = 9.24±3.75) (Table 4). Among all MRSA isolated, 24% were heterogeneous MRSA and 76% were homogeneous MRSA. The numbers of antibiotics resistance (Mean±SD) of homogeneous MRSA (12.21±2.35) were statistically higher than heterogeneous MRSA (6.83±1.47) (Table 5).

Table 3. Multiple antibiotics resistance of MRSA isolated from patients with hospital and community acquired UTI

MRSA	No. (%)	Multiple antibiotics resistance (Mean±SD)
Hospital acquired	34 (68)	11.59±2.69
Community acquired	16 (32)	9.50±3.71
Total	50 (100)	10.92±3.17

Significant (t-value = 2.26, p-value = 0.02).

Table 4. Catheterization and numbers of antibiotics resistance of MRSA isolated from UTI

Urinary catheterization	No. (%)	Multiple antibiotics resistance (Mean±SD)
Catheterization	33 (66)	11.79±2.46
Non-catheterization	17 (34)	9.24±3.75
Total	50 (100)	10.92±3.17

Significant (t-value = 2.89, p-value = 0.006).

Table 5. Heterogeneous and homogeneous MRSA and multiple antibiotics resistance of MRSA isolates from UTI

MRSA	No. (%)	Multiple antibiotics resistance (Mean±SD)
Heterogeneous	12 (24)	6.83±1.47
Homogeneous	38 (76)	12.21±2.35
Total	50 (100)	10.92±3.17

Highly significant (t-value = 7.45, p-value <0.001).

Among 50 MRSA, 41 (82%) were biofilm formation of which 10%, 30%, 42% and 18% exhibited strong, medium, weak, and negative biofilm formation, respectively. Statistically multiple antibiotics resistance of strong biofilm formation MRSA were higher than medium, weak and negative biofilm formation (Table 6). Results found that correlations between: (i) biofilm formation by MRSA with multiple antibiotics resistance, (ii) biofilm formation and heterogeneous MRSA, (iii) biofilm formation and catheterization, (iv) multiple antibiotics resistance of MRSA and homogeneous MRSA, (v) multiple antibiotics resistance of MRSA and catheterization, and (vi) homogeneous MRSA and catheterization (Table 7).

Table 6. Biofilm formation by MRSA and numbers of antibiotics resistance

Biofilm formation	No. (%)	Multiple antibiotics resistance (Mean±SD)
Strong biofilm	5 (10)	13.40±2.51[a]
Medium biofilm	15 (30)	11.93±1.49 [b]
Weak biofilm	21 (42)	11.48±3.11 [b]
Biofilm negative	9 (18)	6.56±1.51 [b]
Total	50 (100)	10.92±3.17

The same letters mean no significant difference, the different letter mean significant difference at p<0.05.

Table 7. Correlations of biofilm formation, multiple antibiotics resistance, homogeneous MRSA and catheterization of MRSA

Factors		Biofilm formation	Multiple antibiotics resistance	Homogeneous MRSA	Catheterization
Biofilm formation	Correlation	1.00	0.57*	0.58*	0.45*
	Significant	.	<0.001	<0.001	<0.001
Multiple antibiotics resistance	Correlation	0.57*	1.00	0.73*	0.39*
	Significant	<0.001	.	<0.001	0.01
Homogeneous MRSA	Correlation	0.58*	0.73*	1.00	0.39*
	Significant	<0.001	<0.001	.	0.01
Catheterization	Correlation	0.45*	0.39*	0.39*	1.00
	Significant	<0.001	0.01	0.01	.

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

DISCUSSION

Microbial biofilms have been associated with a variety of persistent infections, which respond poorly to conventional antibiotic therapy. This also helps in the spread of antibiotic resistant traits in nosocomial pathogens by increasing mutation rates and by the exchange of genes, which are responsible for antibiotic resistance.[27] MRSA were responsible for a significant number of biofilms related infections.[13] The study found that UTI associated with MRSA was high, which is in agreement with other studies,[10,13,23] they found biofilm formation was quite common among clinical MRSA isolates. The reasons for that are MRSA strains colonizes in a hospital and is easily transmitted via skin contact among patients and hospital staff, also increasing number of patients with catheterization.

The increasing prevalence of antibiotic resistant bacteria in hospitals and the community has significantly limited the effectiveness of current drugs resulting in treatment failure.[28,29] In this study, multiple antibiotics resistance among MRSA has been reported that agreement with other studies.[30-35] In addition, biofilm forming by MRSA was correlation with increased antibiotics resistant among MRSA, which is in harmony with other study.[36,37] Such biofilms are responsible for chronic UTIs, which are difficult to treat and show much greater resistance to antibiotics than their planktonic form counterparts.

Complicated urinary tract infection is often refractory to antimicrobial treatment. One of the reasons for this is the fact that the infection is a biofilm disease.[38] Biofilms have been shown to be up to 1000 times more resistant to antibiotics than planktonic cells of the same isolate.[39] The extracellular matrix of the biofilm is not only a passive diffusion barrier for antibiotics but is also actively shaped by species within the microbial biofilm communities, making biofilms extremely difficult to eradicate.[31,40]

The results are similar to other studies,[10,15] they found that significantly greater biofilm forming capacities of MRSA isolates from catheter related cases than of those from catheter unrelated cases. Indwelling medical device associated infections caused by *Staphylococcus aureus* biofilms are significant source of morbidity.[8]

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پوختە

دروست بوونی biofilm بەهۆی خڕۆکە هێشوویە زیرینەکان بەرگریکەر لە مەسسیلین (MRSA) ی دەستکەوتوو لە بۆری میزی تووشبووەکان لە نەخۆشخانەو کۆمەڵگە

پێشەکی و ئارمانج: خڕۆکە هێشویە زیرینەکان بەرکریکەر لە مەسیسلین (MRSA) بەهۆیەکی گرنگ دادەنرێت بۆ تووش بوونی مرۆڤ و تێکدانی ژیانی. وە دەتوانی شێوەکەی بگۆرێ لە شێوەیەکی تاك خانەیی (planktonic) بۆ شێوەیەکی biofilm کە لە زۆربەی کاتدا زۆر بە کەمی لەناو دەبرێن بە دژە زیندەکان بە تایبەتی لەکاتی هەوکردنی درێژ خایەندا.ئەم توێژینەوە لە دروستبوونی biofilm ەکان لە خڕۆکە زێرینە بەرگریکەر لە مەسیسلین دەکۆلێتەوە کە وەرگیراون لەو نەخۆشانەی کە تووش بوون بە هەوکردنی بۆری میز. لە هەمان کاتیشدا بۆ هەڵسەنگاندن و دیاریکردنی پەیوەندنی لە نێوان biofilm ە دروستبووەکان و دژە زیندە بەرگریکەرەکانیاندا.

ریکێن ڤەکولینێ: لە سێ نەخۆشخانەی فێرکاری شاری هەولێر کارەکە ئەنجامدرا بە وەرگرتن و جیاکردنەوەی خڕۆکە هێشوویە زێرینەکان لە بۆری میزی تووشبووەکان بەهەوکردن ئەویش بە رێگەی پێوانەکردن وە بەدیاریکردنی PBP2a. کە heterogeneous MRSA خڕۆکە هێشوویە زێرینە بەرگریکەر لە مەسیسلینی جیاواز دەرکەوتن، بە چالاکی رێگەی رووکەش کردن (efficiency of plating). وە نزمترین ئاستی خەستی بۆ نەهێشتنی دژە زیندەکان دیاری کرا بە رێگەی ئاگاری روون، وە هەروەها biofilm ە دروستبووەکانیش لە خڕۆکە هێشوویە زێرینە بەرگریکەر لە مەسیسلین دیاری کران.

ئەنجام: خڕۆکە هێشوویە زێرینە بەرگریکەر لە مەسیسلین بەرگریکەربوون بۆ 10,92 ± 3,17 لە دژە زیندەکان وە رێژەی سەدی دروستبوونی biofilm ەکان لە خڕۆکە هێشوویە زێرینە بەرگریکەر لە مەسیسلین 82٪ بوو. وە دروستبوونی biofilm ی بەهێز کە بەرگریکەر بوون بۆ 13,40 ± 2,51 لە دژە زیندەکان کە ئەمەش رێژەیەکی بەرز بوو لە چاو دروست نەبوونی biofilm ەکان کە (1,51±6,56 =SD±Mean).

دەرئەنجام: زۆرینەی خڕۆکە هێشوویە زێرینە بەرگریکەر لە مەسیسلین لە شێوەی biofilm بوون، کە ئەم biofilm نەش پەیوەندبوون بە دژە زیندە جیاوازە بەرگریکەرەکانەوە، وە بە خڕۆکە هێشوویە زێرینە بەرگریکەر لە مەسیسلینە جیاوازەکانەوە.

الخلاصة

تشكيل biofilm في المكورات العنقودية الذهبية المقاومة للمثسلين (MRSA) المسببة لالتهاب المسالك البولية المكتسبة من عدوى المستشفى والمجتمع

خلفية واهداف البحث: المكورات العنقودية الذهبية المقاومة للمثسلين (MRSA) هى سبب مهم في العدوى التي تهدد الحياة و التي يمكن ان تتحول من خلايا منفردة (planktonic) إلى biofilms. تشكيل biofilm غالبا ما تكون أقل أستجابة للعلاج بالمضادات الحيوية وتسبب الالتهابات المزمنة. الدراسة تحققت من تشكيل biofilm في المكورات العنقودية الذهبية المقاومة للمثسلين المعزولة من المرضى الذين يعانون من التهاب المسالك البولية. في الوقت نفسه تم تقييم العلاقة بين تشكيل biofilm والمقاومة للمضادات الحيوية.

طرق البحث: تم عزل المكورات العنقودية الذهبية بواسطة الطرق القياسية من التهابات المسالك البولية في ثلاث مستشفيات تعليمية في مدينة أربيل. تم الكشف عن المكورات العنقودية الذهبية المقاومة للمثسلين بواسطة الكشف عن PBP2a. تم تحديد heterogeneous MRSA المكورات العنقودية الذهبية المقاومة للمثسلين بواسطة طريقة efficiency of plating. تم تحديد الحد الأدنى للتركيز المثبطة للمضادات الحيوية بطريقة تخفيف الأكار. تمت الكشف عن قدرة تشكيل biofilm في المكورات العنقودية الذهبية المقاومة للمثسلين.

النتائج: المكورات العنقودية الذهبية المقاومة للمثسلين مقاومة لـ 10,92 ± 3,17 من المضادات الحيوية. النسبة المؤية لتشكيل biofilm في المكورات العنقودية الذهبية المقاومة للمثسلين كانت 82٪. تشكيل biofilm قوي مقاومة لـ 13,40 ± 2,51 من المضادات الحيوية وهى أعلى من الناحية الإحصائية في حال عدم تشكيلها لـ biofilm (=SD±Mean6,56 ±1,51).

الاستنتاجات: معظم المكورات العنقودية الذهبية المقاومة للمثسلين كونت biofilm و تكوينها لـ biofilm ترتبط مع مقاومتها للمضادات الحيوية.

THE EFFECT OF HELICOBACTER PYLORI ERADICATION THERAPY ON PLATELET COUNT IN IDIOPATHIC THROMBOCYTOPENIA: A PILOT STUDY

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ABSTRACT

Background and objectives Conflicting reports on the relationship between *Helicobacter pylori* infection and idiopathic thrombocytopenia had previously appeared in the literatures. This study examines the effect of *Helicobacter pylori* eradication on platelet counts in Iraqi patients with Idiopathic Thrombocytopenic Purpura (ITP).

Methods The study population comprised 31 Iraqi patients with chronic ITP and a platelet count of less than 100.0 $x10^9$/L and positive serum *H pylori* antibodies (indirect immunofluorescence). They were divided into two groups, the first (17 patients) received anti H Pylori plus conventional treatment for ITP, the second group (14 patients) received conventional treatment for ITP only. The effect of *H pylori* eradication on platelet count was evaluated 6 months after therapy.

Results There was significant improvement in platelet count in response to conventional treatment in both groups but there were no significant improvement after H Pylori eradication therapy.

Conclusions Based on this pilot study eradication of *H pylori* does not appear to be effective in increasing platelet count in *H pylori*–positive patients with chronic ITP.

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Key words: Eradication therapy, H. pylori, Immune thrombocytopenia

Helicobacter pylori is a slow-growing, microaerophilic, highly motile, gram-negative spiral bacterial organism etiologically linked to histologic gastritis, peptic ulcer disease, primary B cell gastric lymphoma, and adenocarcinoma of the stomach. A number of other conditions have been suggested as causally related to *H. pylori*, but the data supporting these associations are weak [1]. Two conditions that have increasingly been associated with *H. pylori* and have been assessed by treatment trials are iron deficiency and idiopathic thrombocytopenic purpura[2-4] Immune thrombocytopenic purpura is an acquired disorder leading to immune-mediated destruction of platelets and possibly inhibition of platelet release from the megakaryocyte. The presence of auto-antibodies, often directed against platelet membrane glycoprotein IIb-IIIa, causes the premature removal of platelets by the monocyte-macrophage system. Occasionally, antigen-antibody immune complexes adhere to platelets at their F_c receptor, resulting in their premature removal from the circulation.[5]

The study which is a pilot study was aimed at evaluating the value of *H. pylori* eradication on the outcome of Management of IITP.

METHODS

This is a prospective study conducted in a period between December 2010 and October 2011 in Nanakelly hospital for blood diseases in Hawler, Iraq. During this

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period a total of 106 patients were diagnosed as Idiopathic thrombocytopenic Purpura (ITP). Out of these patients thirty one were *H. pylori* antibody positive. Only the latter group of patients were recruited for the purposes of this study. Eleven were males and twenty were females. Their mean age was 27.3 years (range 7-61). ITP was defined by idiopathic thrombocytopenia (platelets less than 100 x 10^9/L) when other causes had been excluded and with a normal active bone marrow. *H pylori* infection was assessed by the detection of serum antibodies (indirect immunofluorescence).

The recruited patients were divided into two groups. The 1st group comprised 17 patients and received anti-*H. Pylori* eradication plus conventional treatment for ITP, the 2nd group (14 patients) received conventional treatment for ITP only. *H pylori* eradication therapy consisted of amoxicillin (1000 mg twice daily), metronidazole (500 mg 3 times daily), and esmoprazole (40 mg twice daily) for 7 days. Conventional treatment for ITP included prednisolone 1 mg per Kg in cases were platelet count was below 30 x 10^9/L. Platelets counts were monitored every 2 weeks and counts at 6 months post therapy were taken.

Statistical analysis included using t – test. P< 0.05 was considered significant.

RESULTS

Out of the total of 106 Idiopathic thrombocytopenic patients, 31 (29.2%) were H. pylori antibody positive. The latter were divided into two groups, the 1st group (17 patients) who received anti H Pylori plus conventional treatment had a mean +SD platelet count at diagnosis of 28.5 + 29.4 x10^9/L compared to 18.5 + 18.7 x10^9/L for 2nd group who only received conventional therapy (p=0.28).

Following therapy the platelets mean count rose to 107.1 + 57.2 x10^9/L for the 1st group and 128.6 +82.9 x10^9/L for the second group, which again was insignificant (p=0.400).

The mean increase in platelet count in group 1 was slightly lower at 80.2+68.4 x10^9/L compared to group 2, with a mean of 110.2+ 84.5 x10^9/L , however this was not statistically significant (p=0.27).

DISCUSSION

Several lines of direct and indirect evidences suggest that infectious agents may influence the occurrence or the course of some auto-immune diseases.[6-7]

The mechanism by which *H pylori* may play a role in ITP pathogenesis remains unclear. A chronic immunological stimulus induced by *H pylori* or an immune mimicry between platelets and *H pylori* antigens has been suggested as the cause of *H pylori*–induced ITP. [8] Although it has been demonstrated that antibodies against *H pylori* cross-react with human tissues, such as gastric epithelial cells, ductal cells of salivary gland, and renal tubular cells, [9] there is no conclusive support of cross-reactivity with platelets, and conflicting reports on its association with ITP.[10-13]

The prevalence of H. pylori among our ITP patients is comparable to previous studies, including that of Michel and coworkers, who reported a rate 21.6% among 74 patients 10 year or older in age with chronic ITP and platelets count < 60 x10^9/L.[14]

In the present study, we assessed the change in platelet count over time in two groups, there was significant improvement in platelet count in response to conventional treatment in both groups but there was no actual improvement after *H Pylori* eradication. This result is consistent with that of Michel and colleagues who eradicated in *H pylori* in 14 of 15 patients but only one had significant transient platelets improvement.[14] However, Our results is in contrast to those of Gasbarrini et al who documented *H.pylori* infection in

11 of 18 patients; 8 of the 11 patients in whom *H pylori* was eradicated experienced significant platelet increments.[11] Similarly Emilia et al observed *H pylori* in 13 of 30 patients with chronic ITP, and increased platelet counts occurred in 6 of 12 patients following eradication.[15]

H. pylori infection in particular has been recently under intensive clinical investigation. Interestingly, in many countries with a high prevalence of the infection, bacterial eradication reverses the thrombocytopenia in about 50% of cases with chronic ITP. The situation is different in North America, where H. pylori infection is found in a low proportion of cases and eradication seldom has any effect on the platelet count. A plausible explanation is that the *H. pylori* strains differ in different parts of the world.[16] Other possibilities include variations in bacterial virulence factors, such as CagA expression or host immunological class II HLA factors.[17,18] Most Japanese *H. pylori* strains are CagA-positive, unlike most American strains. Patients infected with CagA-positive strains (as measured by anti-CagA IgG antibodies) are thought to have increased platelet response to *H. pylori* eradication compared with those who are CagA-negative or who have low serum titres of CagA antibodies. Serum levels of anti-CagA IgG may be used as a predictor of response to *H. pylori* treatment.[17,18]

In conclusion**,** the results in this preliminary study suggest similar to some previous reports, no significant additional benefis of *H pylori* eradication in ITP. However, further studies including larger number of cases, as well as studies on the prevalence of H pylori among patients with ITP is suggested.

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پوختە

بەکتریایی ئێچ پایلۆری و کەمبوونی خەپلەخوینی نادیار و کاریگەری لە ناوبردنی بەکتریایی ئێچ پایلۆری لە سەر ژمارەیی خەپلەخوینەکان

پێشەکی و ئارمانج: پەیوەندی نیوان پەتابوون بە بەکتریایی ئێچ پایلوری و کەمبوونی خەپلەخوینی نادیار باسکراوە لە لێکۆلینەوەکانی پێشوو. لەم لێکۆلینەوەیەدا جەخت لە سەر کاریگەری لە ناوبردنی بەکتریایی ئێچ پایلۆری لە سەر زیادبوونی ژمارەیی خەپلەخوینەکان.

ریکێن ڤەکولینێ: سی و یەك نەخۆشی تووشبوو بە نەخۆشی کەمبوونی خەپلەخوینی نادیاری درێژخایەن هاتنە دیاریکردن کەهەموویان نموونەی خوینیان پۆزەتیڤبوون بۆ بەکتریایی ئێچ پایلۆری بە رێگایی رەنگدانەوەی ناراستەوخۆ (Indirect immunofluresence). نەخۆشەکان کران بە دوو گرووپ، گرووپی یەکەم (17 نەخۆش) هاتنە چارەسەرکردن بە دژە بەکتریایی ئێچ پایلۆری لەگەل چارەسەرکردنی تەقلیدیی کەمبوونی خەپلەخوینی درێژخایەن، وە گرووپی دووەم (14 نەخۆش) هاتنە چارەسەرکردن تەنها بەرێگە چارەی تەقلیدیی. لە شەش مانگ دا بەدواداچوونی حالەتەکان کرا.

ئەنجام: ئامارەکان ئەوە نیشان دەدەن کە لەناوبردنی بەکتریایی ئێچ پایلۆری رۆلێکی گرنگی نیە بۆ زیادکردنی ژمارەیی خەپلەخوینەکان.

دەرئەنجام: لە ناوبردنی بەکتریایی ئێچ پایلۆری هیچ رۆلێکی نیە لە بەرزکردنەوەیی ئاستی خەپلەخوینەکان لە ئەو نەخۆشانەیی کە توشبوون بە کەمبوونی خەپلەخوینی درێژخایەن.

الخلاصة

تأثير استئصال جرثومة الهليكوباكتر على عدد الأقراص ألدموية لدى المرضى المصابين بنقص الأقراص ألدمويه الفرفريه العفوي

خلفية واهداف البحث: العلاقة بين الهليكوباكتر ونقص الأقراص ألدمويه الفرفريه العفوي مثبته سابقا. هذه الدر اسه تحدد تأثير استئصال جرثومة الهليكوباكتر على عدد الأقراص ألدمويه.

طرق البحث: هذه الدر اسه تتضمن واحد وثلاثون مريض عراقي مصابون بمرض نقص الأقراص ألدمويه الفرفريه العفوي مع عدد الأقراص اقل من 100000 مصابون بجرثومة الهليكوباكتر.المرضى قسموا إلى مجموعتين ألمجموعه الأولى (17) استلمت علاج استئصال جرثومة الهليكوباكتر مع العلاج التقليدي لمرض نقص الأقراص ألدمويه الفرفريه. ألمجموعه ألثانيه استلمت العلاج التقليدي لمرض نقص الأقراص ألدمويه الفرفريه فقط . تأثير استئصال جرثومة الهليكوباكتر على عدد الأقراص ألدمويه تم تقييمه لمدة 6 أشهر.

النتائج: كان هناك تحسن معتبر بعدد الأقراص ألدمويه في المجموعتين ردا على العلاج التقليدي ولكن لم يكن هناك تحسن معتبر بعد استئصال جرثومة الهليكوباكتر.

الاستنتاجات: استئصال جرثومة الهليكوباكترغير فعال في زيادة عدد الأقراص ألدمويه عند مرضى نقص الأقراص ألدمويه الفرفريه المصابون بجرثومة الهليكوباكتر.

METHYLENETETRAHYDROFOLATE REDUCTASE (C677T) MUTATION IN HEALTHY INDIVIDUALS IN ERBIL - IRAQ

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ABSTRACT

Background and objectives Methylenetetrahydrofolate reductase is one of the main regulatory enzymes of homocysteine metabolism. A 677C→T mutation in the methylenetetrahydrofolate reductase gene results in decreased enzymatic activity, and contributes to increased plasma homocysteine. The association between the C677T mutation in the methylenetetrahydrofolate reductase gene and vascular disease is controversial, and may be affected by ethnic origin. The aim of this study was to study the frequency of the C677T methylenetetrahydrofolate reductase mutation in healthy individuals from Erbil-Iraq.
Methods A total of 100 healthy individuals attending the premarital screening center in Erbil city were recruited. methylenetetrahydrofolate reductase (C677T) gene polymorphism was investigated in all of them by the polymerase chain reaction and restriction fragment length polymorphism.
Results Methylenetetrahydrofolate reductase C677T was documented in homozygous and heterozygous state in 6% and 37% respectively.
Conclusions Methylenetetrahydrofolate reductase C677T mutation is commonly encountered among healthy individuals in Erbil city, although it was rather less frequent than that documented earlier in Duhok, to the north of the former city. The clinical implications of our finding require further clinical studies.

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Key words: C677T *MTHFR* gene mutation, PCR-RFLP, Duhok - Iraq

The methylenetetrahydrofolate reductase (MTHFR) gene, located on the short arm of chromosome 1 (1p36.3), presents two common polymorphisms involving nucleotides C677T and A1298C. The change of C for T at position 677 causes the substitution of alanine for valine in the MTHFR protein and the consequent reduction in enzyme activity. The specific activity of the MTHFR enzyme is reduced by 35% in the presence of heterozygous, genotype C/T, compared to the normal genotype C/C, and by 70% in homozygous, genotype T/T.[1] MTHFR is an enzyme in the transmethylation pathway where homocysteine (Hcy) is converted to methionine, thus impaired enzyme activity leads to hyperhomocysteinemia.[2] MTHFR enzyme has important role in metabolic pathway of folate and nucleotide methylation.[3] Presence of T allele at position 677 of MTHFR gene leads to reduction of MTHFR activity and DNA hypomethylation. Genetic variation in this gene influences susceptibility to occlusive vascular disease, neural tube defects, colon cancer and acute leukemia, and mutations in this gene are associated with methylenetetrahydrofolate reductase deficiency.[4]

There is ethnic variability in the frequency of the T allele–frequency. The latter in Mediterranean/Hispanics is greater than the frequency in Caucasians which, in turn, is greater than in Africans/African-Americans.[5] Mutations of the methylenetetrahydrofolate reductase (MTHFR) gene have been shown to be associated with a predisposition to developing diabetic nephropathy (DN) in specific populations.[6] Elevated levels of

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total plasma homocysteine have been linked to increased all-cause mortality,[7] arteriosclerosis,[8] and thromboembolism.[9,10] This study was aimed to determine the prevalence of MTHFR mutation in healthy individuals In Erbil northern Iraq. Determining such prevalence rates and comparing the data with those found in other regions.

METHODS

The total studied sample consisted of 100 apparently healthy individuals attending the premarital screening center in Erbil for routine mandatory checkup and included 56 male and 44 female and with age ranges of 16-45 and 15-38 years respectively. From each individual 3 mL of whole blood were collected in EDTA for isolation of genomic DNA using a phenol chloroform method.[11]

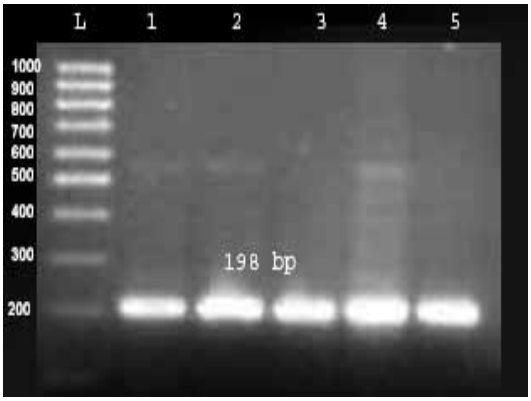
The C677T *MTHFR* gene mutation was detected by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) analysis using *Hinf* I restriction enzyme according to Frosst et al.[8] The PCR reactions were done in a sterile 0.2 ml tube, the reaction mix was of a final volume of 25 µL containing 1×PCR buffer (Promega – USA) with 1.5 mmol/L $MgCl_2$, 1.5 unit Taq DNA polymerase, 10 µmol/L dNTP, DNA template 2.5 µl, primers (5'-TGAAGGAGAAGGTGTCTGCGGGA-3' and 5'-AGGACGGTGCGGTGAGAGTG-3') (0.5µl each), completed to 25 µl with sterile distilled water. PCR Conditions for *MTHFR 677*: Pre-PCR: 94°C for 8 Min, then 40 cycles of: denaturation:94°C for 1 min, annealing:63°C for 1 min, extension :72°C for 1 min, followes by final extension for 7 min.[12] PCR products were checked on 2% of agarose gels followed by staining with ethidium bromide to stain DNA fragments. The amplified PCR products (MTHFR) were subjected to *Hinf* I restriction enzyme digestion at 37°C overnight. Digestion was carried out in a final volume of 10µL, using 8.5µL of PCR product, 5 units of *HinfI* enzyme, and 1.0µL of buffer. Size analysis of the restriction fragments were visualized by gel electrophoreses of digested PCR products using 3% agarose and stained with ethidium bromide. Polymorphism C677T creates a recognition sequence for the restriction enzyme *Hin*fI, and this is detected by digestion of the 198-bp PCR product, generating 23- and 175-bp fragments for the polymorphism in homozygous state (genotype TT). Genotype CC was characterized by the presence of a 198-bp fragment, and genotype CT was characterized by the presence of three fragments, 198 bp, 175 bp and 23 bp.[13]

RESULTS

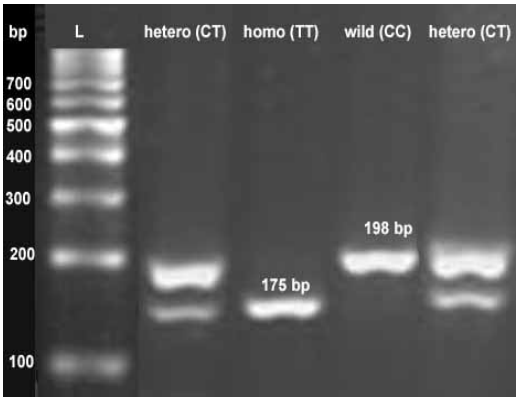
Amplification using specific primers on DNA extracted on the 100 apparently healthy individuals yielded a 198 bp product. After restriction digestion of PCR products with *Hinf* I: 6 samples (6%) had full digestion yielding 175 bp (the 23 bp was too faint to be detected on agarose gel) and thus were labeled as homozygous (TT genotype), 37 (37%) had the 175 in addition to the 198 bp fragments, thus labeled as heterozygous (CT genotype), while the remaining 57 Ampicons (57%) did not show any digestion and just retained the original 198 kb fragment, and thus labeled as Wild genotype (CC) (Table 1) and (Figure 1).

Table 1. The number and frequency MTHFR 677CT genotype mutations found in healthy individuals from Erbil –Iraq (100 healthy individuals)

Sex of individuals	Homozygous (TT) No. (%)	Heterozygous (*CT*) No. (%)	Wild (*CC*) No. (%)
Males (n= 56)	4 (7)	23(41)	29 (51.8)
Females (n= 44)	2 (4.5)	14(31.8)	28 (63.6)
Total	6(6)	37(37)	57(57)



A



B

Figure 1. A. Represent the PCR product MTHFR C677T genotype bands in 198bp fragments. Polymorphism analysis of the methylenetetrahydrofolate reductase gene (MTHFR) amplicons by agarose gel electrophoresis after restriction endonuclease digestion (RFLP). CT genotype yields 198bp and 175bp TT genotype (175bp), and CC (198bp). L: Ladder molecular weight.

DISCUSSION

Erbil (Hewlêr in Roman-alphabet Kurdish) (also written Irbil) is a large city in northern Iraq with a population of approximately 1.3 million (2009), bordering Turkey to the north and Iran to the east. It is largely populated by Kurds but has a small minority of Assyrians.

This study showed that the frequency of homozygosity to MTHFR C677T mutation was 6% which is lower than that reported in previous study from Duhok to the north of Erbil, where a frequency of 8% was found.[14] Table 2 demonstrates that the frequency of homozygosity to MTHFR C677T mutation was 6% also lower than those reported from neighboring Syria,[15] Turkey,[16] Jordan,[17]Tunisia,[18] some European countries,[19] Brazil,[13] USA,[20,21] Japan,[22]china,[23] and Korea.[24]

However it was higher than that reported from Iran,[25] Oman,[26] Bahrain,[27] Egypt,[28] Northern Italy,[29] Pakistan,[30] and North India.[31] However and in contrast to the above reports, homozygosity for this polymorphism is almost absent in Africans and people of African descent.[21,22]

A study confirmed a relatively high frequency of the 677TT genotype in the French population and an association with elevated tHcy concentration in men but not in women.[32] Individuals homozygous for the C677T mutation have moderately increased concentrations of fasting plasma homocysteine especially in the presence of low (<15.4 mol/L) plasma folate. [33] The current study did not however include studying homocysteine levels in those with the mutation, which may have added valuable information.

Several previous studies revealed that a very common mutation in the MTHFR gene C677T is related to mild homocysteinemia and might increase the risk for vascular occlusive pathology. However, other recent publications negate this relationship.[34,35,36] In their important study, Frosst and colleagues not only identified the MTHFR mutation responsible for thermolability of the enzyme but also established that subjects homozygous for this mutation had elevated fasting and postmethionine plasma homocyst(e)ine concentrations.[8] The association between the mutation and elevated fasting homocyst(e)ine was confirmed also by van der Put and colleagues in 1995, who found that the mutation was associated with decreased MTHFR activity.[37] Homozygosity for the mutation, and to a lesser extent heterozygosity, were associated with moderately increased fasting tHcy levels.[38-40]

Table 2. The frequencies of the MTHFR (C677T) Homozygous (TT) % mutation in Eastern Mediterranean and worldwide studies

Location	MTHFR (C677T) Homozygous (TT) %	References
Iraq/ Erbil	6	Current study
Iraq/ Duhok	8	14
Turkey	9.6	16
Iran	5	25
Syria	18	15
Jordan	8	17
Oman	2.45	26
Bahrain	2.6	27
Egypt	5	28
Tunisia	7	18
Europe	8-18	19
USA	13	20,21
Brazil	9.6	13
Africa/African origin	0	21,22
Japan	11.5	22
Pakistan	1	30
China	17.8	23
North India	1.5	31
Northern Italy	4	29
Korean	14.05	24

The study of Peadar, and coworker revealed that heterozygosity for the MTHFR polymorphism, which is present in 38% of the population, increases the risk of neural tube defects. Most studies of MTHFR C677T and neural tube defects and other conditions have focused on the risk associated with T allele homozygosity. The possibility that heterozygosity might also increase neural tube defect risk has gone unrecognised except for a small study in which an association between CT and these malformations was thought to be due to the higher than expected proportion of CC control subjects.[41] The individuals with MTHFR C677T C/T and T/T genotypes were at a higher risk of developing colon cancer, but they were at a decreased risk of developing rectal cancer.[42,23] Polymorphism at C677T shows marked heterogeneity based upon ethnicity and geographical location.[43] Only individuals who were homozygous (TT) for the C677T mutation had significantly higher plasma total homocysteine concentrations, which is in accordance with what has been reported previously.[8,38] The data demonstrate that 677C T polymorphisms, whether homozygous or heterozygous, are significantly associated with anemia sickle disease (ASD). The homozygous (TT) individuals are reported to have an approximately 50% decrease in MTHFR enzyme activity, and the heterozygous (CT) a 30% decrease in enzyme activity as measured in their lymphocytes.[21]

Finaly methylenetetrahydrofolate reductase C677T mutation is commonly encountered among healthy individuals in Erbil city, although it was rather less frequent than that documented earlier in Duhok, to the north of the former city. The clinical implications of our finding require further clinical studies.

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پوختە

گهورینا جینێ C677T تێتراهایدروفولێت ریدەکتێز MTHFR دناڤ کەسێن ساخلەمدا ل هەولێرێ – عیراق

پێشەکی و ئارمانج: ئەنزیمێ تێتراهایدروفولێت ریدەکتێز MTHFR ئێکە ژ ئەنزیمێن سەرەکی یێن رێکخەر بو میتابولیزما هوموسستینی. ئێك ژ گهورینێن مشە د جینێ قی ئەنزیمی دا گهورینا C→T677 کو دبینە ئەگەرێ کێمبوونا کارێ ئەنزیمی و بلندبوونا رێژا هوموسستینی د خوینێ دا.هەڤبەندی دناڤبەرا ڤێ گهورینێ دا و نەخوشیێن دەمارا باش یا دیار نینە, و دبیت کارلێکرن هەبیت ژ بنەمایێ نژادی ڤە. ئارمانجا ڤێ ڤەکولینێ دیارکرنا مشەتیا ڤێ گهورینا جینی دناڤ کەسێن ساخلەم دا ل هەولێرێ – عیراق.

ریکێن ڤەکولینێ: سەرجەمێ 100 کەسێن ساخلەم یێن سەرەدانا سەنتەرێ پشکنینێن بەری مارەبرینێ ل هەولێرێ کری هاتنە بەشدارکرن دڤەکولینێ دا. فرەشێوەییا جینێ MTHFR (C677T) هاتە پشکنینکرن بو هەمی بەشداربوویا بریکا PCR و RFLP بو پارچا DNA ل نافکوکا 677 بو هەمیان.

ئەنجام: گهورینا MTHFR C677T هاتە دیتن بشێوێ ئێك جور ل 6٪ و هەمەجور ل 37٪ ژ بەشداربوویا.

دەرئەنجام: گهورینا MTHFR C677T مشە دهێتە دیتن لدەف کەسێن ساخلەم ل هەولێرێ, هەرچەندە رێژە کێمترە ژ یا بەری نوکە هاتیە دیتن لدهوکێ لباکورێ هەولێرێ. کارتێکرنێن کلینیکی یێن ڤێ ڤەکولینێ پێدڤی ب پتر ڤەکولینایە.

الخلاصة

الطفرة في جين C677T (Methylenetetrahydrofolate reductase MTHFR) في الاصحاء في مدينة اربيل – العراق

خلفية واهداف البحث: يعد انزيم (MTHFR methylenetetrahydrofolate reductase) احد الانزيمات الرئيسية المنظمة لايض ال homocysteine . ان الطفرة A 677C→T في جين MTHFR ينتج انخفاض في الفعالية الانزيمية و هذا يساهم في زيادة homocysteine في البلازما. العلاقة بين الطفرة C677T في جين ال MTHFR المرض الوعائي لاتزال غير مؤكدة وربما تتاثر بالعرق. نهدف الدراسة الحالية الى احتساب تردد الطفرة C677T في انخفاض الاصحاء في مدينة اربيل / عراق.

طرق البحث: جمعت عينات من 100 شخص اصحاء متطوعون من المراجعين الى مركز الفحص قبل الزواج في مدينة اربيل–العراق. تم تقدير تعدد اشكال الجين الطافر C677T MTHFR في كل العينات باستعمال تقنيات ال PCR–RFLP.

النتائج: وجد أن نسبة الجين الطافر C677T MTHFR في متماثل الزيجة 6% بينما في متباين الزيجة 37%.

الاستنتاجات: اتضح ان تردد الطفرة في جين C677T MTHFR شائعا في الاشخاص الاصحاء في مدينة اربيل وبالاحرى كان هذا التردد اقل من تردد نفس الجين في دراسات مبكرة في مدينة دهوك شمال مدينة اربيل. ان النتائج السريرية الحالية تحتاج الى دراسات سريرية اكثر.

CHILDHOOD HENOCH-SCHÖNLEIN PURPURA IN KURDISH POPULATION OF DUHOK CITY

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ABSTRACT

Background and objectives Henoch-Schönlein purpura is the most common systemic vasculitis in children, mainly affecting the skin, joints, gastrointestinal tract, and kidneys. This study was designed to assess the clinical and epidemiological characteristics of Kurdish children with Henoch-Schönlein purpura in Duhok city.

Methods A prospective study was conducted on children diagnosed as Henoch-Schönlein purpura, who were treated in Heevi pediatric teaching hospital between July, 2009 and July, 2011. The collected data included age, sex, the initial presenting feature, associated systemic clinical features, season of presentation, and associated triggering factors. Laboratory investigations included complete blood count, erythrocyte sedimentation rate, renal function tests and urinalysis. Skin biopsy was taken from 11 patients. Patients were followed up by urinalysis and renal function tests for 6 months to pick up renal involvement.

Results Over the 2 years period, a total of 51 patients were diagnosed with Henoch-Schönlein purpura, of whom 28 were males and 23 females. The patients were aged between 10 months-15 years, the mean age at presentation was 7.1 years, with a male to female ratio of 1.2:1. Disease onset was more common in winter and autumn. All cases had palpable purpura. Large joint arthritis/arthralgia occurred in 41 (80.4%), abdominal pain in 24 (47%) patients, and renal involvement in one patient. The result of skin biopsy was consistent with the diagnosis of Henoch-Schönlein purpura. Complete recovery occurred in all patients. After 6 months of follow-up, no patient had renal involvement.

Conclusions Henöch-Schonlein purpura in Kurdish children is milder with fewer renal manifestations than that in the previously published studies in other areas.

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Key words: Henoch-Schönlein purpura, Purpura, Arthritis, Skin biopsy

Kawasaki disease, most practicing pediatricians will never encounter a case.[1] HSP is a systemic small vessel vasculitis characterized by non-thrombocytopenic palpable purpura, arthritis, bowel angina and hematuria/proteinuria.[2-4] Although HSP is a condition that can occur from age 6 months to adulthood, 50% of cases occur in children under 5 years of age and 75% are under 10 years. In most reports HSP is more common in boys.[5] This disease is usually self-limiting. Involvement of internal organs such as kidney, intestine and central nervous system are the major complications.[2-4] The prognosis is thought to be good as long as the patients have no renal symptoms.[6]

Although HSP is not uncommon in children, there are few large-scale epidemiological studies of childhood HSP, especially nationwide surveys.[7-9] There is no existing data in Kurdistan region (North of Iraq) on this topic.

In this study, we prospectively evaluated the epidemiological and clinical

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data, main laboratory abnormalities and outcome in 51 Kurdish children with HSP followed by a single center, over a follow-up period of 6 months.

METHODS

All Kurdish children with HSP who were diagnosed by pediatricians and treated in Heevi Hospital in Duhok city, between July 2009 and July 2011included in this study (Non-Kurdish children were excluded). The diagnosis was made based on the American College of Rheumatology criteria.[10] Patients were diagnosed as HSP if they had palpable purpura not related to thrombocytopenia, with or without other manifestations. An atypical HSP case was defined as a HSP patient without skin rash in the first 24 hours of admittance.[11] The demographic (age and sex), and clinical (recent history of febrile illness, seasons of occurrence, presence of purpura, joint involvement, gastrointestinal manifestations, renal involvement, other organ involvements) characteristics were collected and analyzed.

Laboratory investigations included complete blood count (CBC) and erythrocyte sedimentation rate (ESR), blood urea and serum creatinine. In addition, repeated urinalyses were undertaken to detect renal involvement. HSP nephritis (HSN) was defined as the presence of gross or microscopic hematuria with or without proteinuria.[12] The following definitions were adopted for renal involvement: microscopic hematuria (> 10 erythrocytes/ HPF), macroscopic hematuria (> 100 erythrocytes/HPF), proteinuria (> 5 mg/kg/24 h).[12] Ultrasound of the abdomen was done in all patients with abdominal pain.

Skin biopsy from the involved skin was taken from 11 patients and subjected to light microscopy. The patients were followed up every 2 weeks for the first 3 months and every month for the next 3 months by urinalysis and renal function tests. This study received prior approval from the Medical Ethics Committee at the College of Medicine, University of Duhok.

RESULTS

Over the 2 years period, a total of 51 patients were diagnosed with HSP, of whom 28 (54.9%) were males and 23 (45.1%) females and the male-to-female ratio was 1.2:1. The age at presentation ranged between 10 months to 15 years old, and the mean age at presentation was 7.1 years. Figure 3.1 shows the distribution of number of cases according to patients' age and sex. Most cases presented between 5 and 10 years (n=45; 88.2% of total cases).

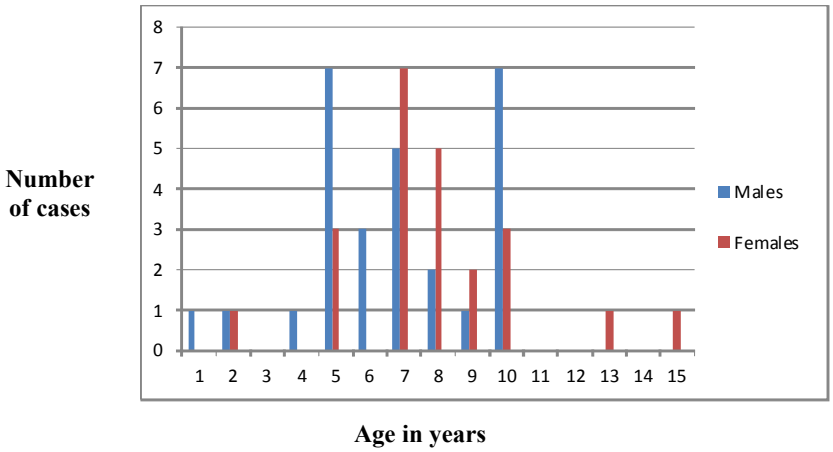


Figure 1. Age and sex distribution of patients

Figure 3.2 shows that HSP could occur year-round, but more commonly in winter and autumn than in other seasons. The clinical data of patients are shown on table 3.1.

Thirty-three (64.7%) patients had history of respiratory infection about 1-3 weeks before the onset of the disease. The presenting symptoms were skin rash (Figure 3.3) in 51 (100%), joint symptoms in 41 (80.4%) and abdominal pain in 24 (47%) patients. The mean duration of symptoms before diagnosis was 2 days (range 1-4 days).

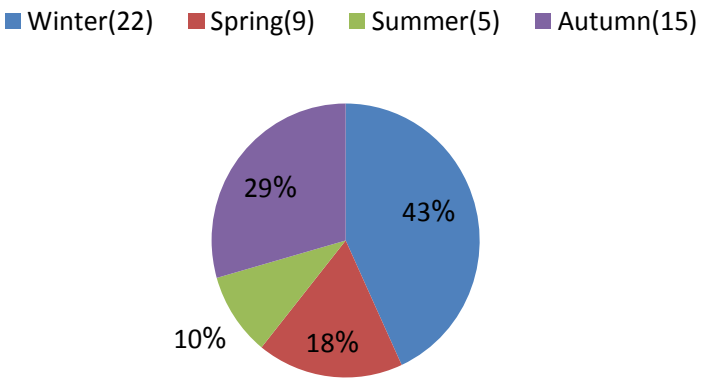


Figure 3.2 Seasonal variations in Henoch-Schönlein purpura cases

Table 3.1. Clinical data of patients (n=51)

Clinical data	No. (%)
History of respiratory infection	33 (64.7)
Purpura	51 (100)
Lower limb	51 (100)
Buttocks	29 (56.8)
Upper limbs	21 (41.1)
Face	1 (1.96)
Arthritis/Arthralgia	41 (80.4)
Abdominal pain	24 (47)
Melena	1 (1.96)
Edema of extremities	13 (25.5)
Microscopic hematuria	1 (1.96)
Fever	8 (15.6)
First manifestation	
Purpura	42 (82.3)
Arthritis/Arthralgia	5 (9.8)
Abdominal pain	4 (7.8)

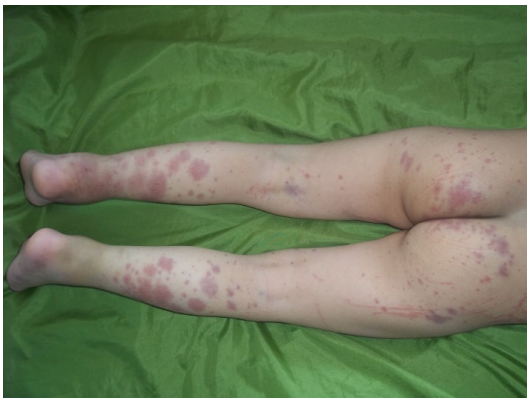


Figure 3.3. The classic rash of Henoch-Schönlein purpura

The variability of cutaneous morphology was noted in one patient. This variation was found in a 9 year old girl who presented with hemorrhagic vesicles and bullae varying in size from 10 to 30 mm in diameter, on both legs, and dorsum of feet, in addition to palpable purpuric rash (Figure 3.4).

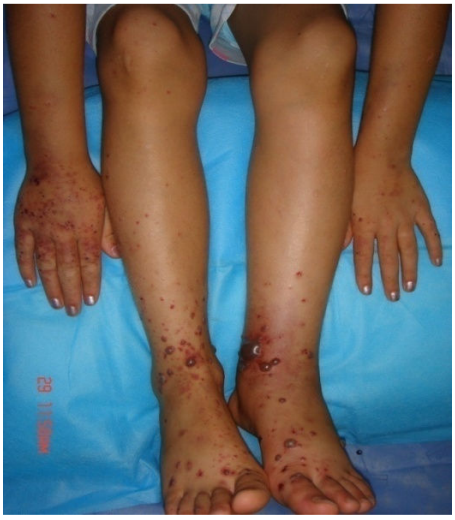
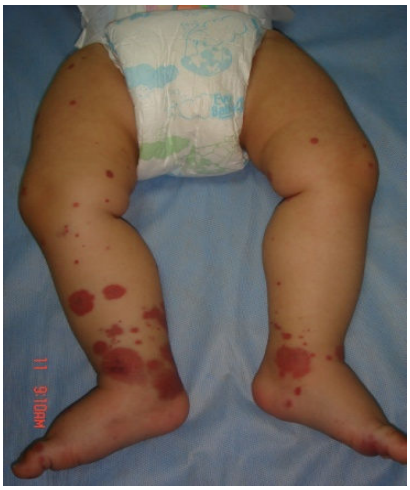


Figure 3.4. Hemorrhagic vesicles, a rare presentation of Henoch-Schönlein purpura. Skin biopsy demonstrated leukocytoclastic vasculitis

One patient (1.96 %) was infant (10 months old boy), and was admitted for a purpuric rash observed 1 day earlier. At the time of admission he was febrile and had a purpuric rash on his face, pinnae, and extremities. His hands and legs were edematous (Figure 3.5 A, B, and C).



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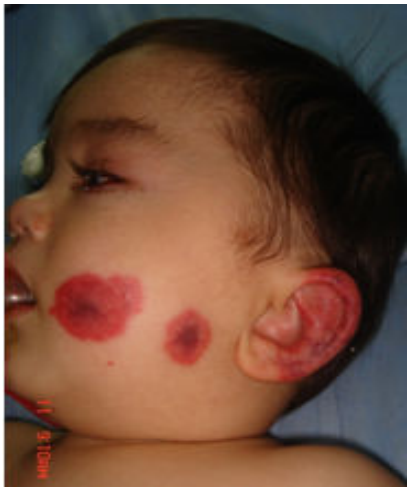

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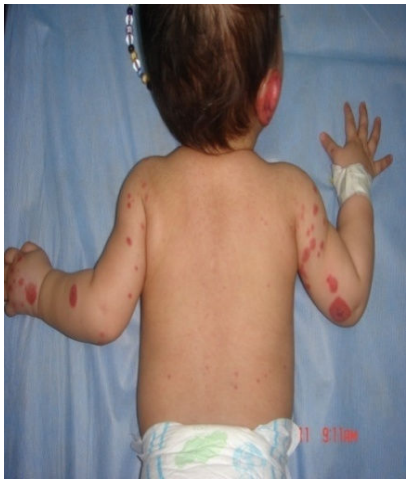

C

Figure 3.5 A, B, and C. An infant with Henoch-Schönlein purpura on face, ears, and extremities. Skin biopsy demonstrated leukocytoclastic vasculitis

Only one patient (1.96%) had microscopic hematuria without proteinuria and normalization of urinary abnormalities occurred over a period of 2 weeks. No cases had central nervous system or pulmonary manifestations.

Elevated ESR (>30 mm/h) was observed in 16 (31.3%) patients, and mild thrombocytosis (450-500 x 10^9 /L) in 19 (37.2%) patients. Other blood elements were normal. Renal function tests were normal, even in the patient with hematuria.

Ultrasound abdomen was normal in all patients with abdominal pain.

The skin biopsy was performed in 11 patients (one was an infant, one had hemorrhagic vesicles, 5 had skin and joint manifestations, and 4 had skin and GIT manifestations). Light microscopy of skin biopsy showed perivascular infiltrate of polymorphnuclear leukocytes, nuclear dust, extravascular erythrocytes, and fibrinoid necrosis of the vessel walls. These findings are consistent with leukocytoclastic vasculitis. Direct immunofluorescence examination to reveal the presence of IgA and C3 in the mesangium and capillary wall was not done (not available).

The average duration of the disease was 9 days (range 6-14 days) and all patients recovered uneventfully. None of the patients who escaped renal involvement at initial presentation have developed any urinary abnormalities on follow-up. Only 3 patients were lost to follow up after one month.

DISCUSSION

Although few cases of HSP might have been diagnosed and treated elsewhere (e.g. dermatology or private clinics), we have seen 51 cases of HSP over a period of 2 years. To our knowledge, the present study is the first one from Iraq where the clinical diagnosis has been confirmed by histopathological study on skin biopsy in some of the patients. The diagnosis of HSP in this series was made according to the American College of Rheumatology criteria,[10] though a new and more realistic criteria, EULAR/PreS endorsed consensus criteria for classification of childhood vasculitides,[13] has been proposed. We believe that both methods can be applied to our patients with the same results, because all patients had joint and/ or GIT manifestations in addition to skin involvement, and no patient showed isolated palpable purpura (i.e., without other features).

In this study, the mean age of patients at presentation (7.1 years) and the male-to-female ratio (1.2:1) were comparable to that reported from Turkey,[11] Jordan,[14] India,[15] and Taiwan,[16] while some studies have shown a female predominance.[17,18] These variations may be attributable to the small sample sizes in the majority of previous studies and different time frames, races and geographical areas from which the data were recorded and analyzed.

The great majority of our patients (72%) presented during winter and autumn, and 64.7% of patients had a respiratory infection prior to onset of the disease. A similar seasonal pattern was noticed by other authors.[11,14,16] The disease clustering in winter and the histories of preceding upper respiratory infections recorded in many HSP patients,[4,11,19,20] provide clues to the possibility that HSP is infection-related, but drugs (antibiotics, ACE inhibitors, NSAIDs) and certain toxins (insect bites, vaccinations and food allergies) have also been implicated.[18,21] These epidemiological characteristics may be valuable for disease prevention and for the further etiological studies of HSP.

All patients in this study had skin involvement (purpura), which appeared as the first manifestation at onset of disease in 82.3%, while abdominal pain and arthritis preceded the rash in 4 (7.8%) and 5 (9.8%) patients, respectively. This is in contrast to the results reported by Kumar *et al*.[15] where the rash appeared as the first manifestation in 47% and abdominal pain preceded the rash in 29% patients.

Occurrence of severe acute abdominal pain in the absence of skin rash may lead to misdiagnosis of acute abdomen and unnecessary surgical exploration.[21,22]

One patient was found to have hemorrhagic bullae and vesicles, in addition to purpuric rash. Bullous eruption in HSP, as in our case, appears often in adults while it is very rare in children.[23] Clinicians must be aware that HSP can present with atypical features that make the diagnosis difficult.

Transient arthritis/arthralgia involving large joints and GIT involvement (abdominal pain) were comparable to that reported in other studies,[18,19,21,24,25] though serious GIT complications (e.g., hemorrhage, perforation and intussusceptions) were absent.

The youngest child among our patients was 10 months old boy, who was the only patient under 2 years of age in our sample. The occurrence of HSP in infants is considered rare.[21,26] However, infantile hemorrhagic edema has been described, and the question is asked whether this is a very early form of HSP or a different vasculitis altogether.[27,28]

Renal involvement (nephritis) is potentially the most worrisome feature of HSP, and the majority of these (85%) doing so within the first 4 weeks and 97% within six months.[29] Only one of our patients (1.96%) had microscopic hematuria during the acute stage of the disease, in contrast to the higher incidence reported by other authors (13.6-45%).[11,14,15,21,30]

After 6 months of follow-up, no patient had renal involvement. There is no need to follow up after the first six months those whose urinalysis remains normal, but measurements of serum urea and creatinine need to continue in the presence of continued urinary abnormalities.[29]

According to other studies in the medical literature,[31-34] renal involvement is the principal determinant of severity and prognosis in HSP, therefore, the lower rate of renal involvement in our patients is an indicator of mild pattern of the disease and it suggests ethnic differences for nephritis in HSP, which was reported by other authors.[35]

In the pediatric population, skin biopsy is reserved for patients with an unusual presentation of HSP (no rash or atypical rash) and patients with significant renal disease.[36] However, skin biopsies have been suggested as possible diagnostic criteria for HSP, and are commonly used as such in the case of adults.[37] Owing to the typical presentation of our patients, we performed skin biopsy in 11 cases. The light microscopy with hematoxylin and eosin stains demonstrated a classic leukocytoclastic vasculitis in postcapillary venules in the 11 patients.

Elevated ESR and thrombocytosis are well documented features of HSP,[18,38] and were seen in 31.7% and 46% of our patients, respectively. Thrombocytosis helps in distinguishing this form of purpura from that caused by thrombocytopenia.[38] The degree of thrombocytosis is believed to correlate with severity of illness,[5,39] though this could not be documented in our patients.

The frequency of relapses varies from series to series. Relapses occurred in 33% of American patients,[7] in 5% of Turkish patients,[11] in 15% of Spanish patients,[18] and in 35% of Italian patients.[40] In our study group, all patients recovered uneventfully and neither chronicity nor relapse was recorded during the period of follow up (6 months). However, patients with HSP should be followed over the long term, since recurrence is likely to occur, especially during a 2-year period after the first outbreak.[4,25] The absence of relapse of HSP in our patients may be explained, to some extent, by mild pattern of the disease and absence of renal involvement which are regarded as possible predictive factors for relapse.[33,34]

In conclusion, the demographic and epidemiological characteristics of HSP in Kurdish children are comparable to that reported from other countries, but it is

milder with lower rate of renal involvement. It appears that ethnic differences play a role in the presentation pattern of HSP. Larger studies are required to confirm the results in this study, to monitor the long-term outcome of the disease and to compare with other ethnic groups in the same area.

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پوختە

نەخوشیا پرسکێن هینوك– شونلاین لدەف زاروکێن کورد ل باژێرێ دهوکێ

پێشەکی و ئارمانج: پرسکێن هینوك– شونلاین مشەترین نەساخیا دەمارێن خوینێ یە کو توشی پیستی وگەها و کوئەندامێ هەرسکرنێ و گولچیسکا ل ناف زاروکا. ئەڤ ڤەکولینە هاتە کرن ژبو خواندنەڤەیا لایەنێ کلینیکی یێ نەخوشیا پرسکێن هینوك– شونلاین لدەف زاروکێن کورد و هەروەسا ژبو دانا رونکرنەکێ لسەر لایەنێ دەردزانی یێ ڤێ نەخوشیێ لباژێرێ دهوکێ– باکورێ عیراقێ.

ریکێن ڤەکولینێ: بو ڤێ ڤەکولینێ ئەو زاروکێن نەخوشیا پرسکێن هینوك– شونلاین لێ هاتیە دەستنیشانکرن و چارەسەرکرن ل نەخوشخانا هیڤی یا زاروکان هاتنە وەرگرتن دماوێ دناڤبەرا چریا ئێکێ 2009 هەتا تیرمەها 2011ێ. نەخوش هاتنە هەلسەنگاندن ژلایێ ژی, رەگەز, نیشانێن دەستپێکی, هەبوونا نیشانێن دی یێن کلینیکی, وەرزێ نەساخیی و فاکتەرێن دی یێن پەیوەندیدار. و بو هەر نەخوشەکی ئەڤ تاقیکرنە هاتنە کرن: CBC,ESR و تاقیکرنێن شولێ گولچیسکا و تاقیکرنا دەستاڤا زراڤ. نمونەك ژ پیستی هاتە وەرگرتن ژ 11 نەخوشا.

ئەنجام: لسەرانسەری دو سالێ ڤەکولینێ 51 زاروك هاتنە دەستنیشانکرن کو ئەڤ نەخوشیە هەی, و ژوان 23 کچ بوون و 28 کور. ژیێ وان دناڤبەرا 10 هەیڤی هەتا 15 سالی بووو تێکرایێ ژیێ وان 7.1 سال بوون. دەستپێکرنا نەخوشیێ پتر ل وەرزەی زستانێ وبابیزی بوو. هەمی نەخوشان پرسکێن بدەست هەستیار هەبوون. کولبوون وئێشانا گەهێن مەزن لدەف 41 (80.4٪) زاروکان هەبوو و ئێشانا زکی لدەف 24(47٪), بەلێ توشبوونا گولچیسکێ بتنێ لدەف ئێك زاروك هەبوو.

دەرئەنجام: نەخوشیا پرسکێن هینوك–شونلاین ئێك ژ نەخۆشییێن بەربەلاڤە دەڤ زاروکێن مە بەس سڤکترە و زۆر کێم گولچیسکان ڤەدگریّت.پێتڤییە چاڤدێریا دریژ بۆ تووش بویا بهێتە کرن بۆ تۆمارکرنا ئەوێن دوجارکی تووش دبن.

الخلاصة

فرفرية هينوك–شونلاين لدى الأطفال الكرد في مدينة دهوك

خلفية واهداف البحث: فرفرية هينوك–شونلاين من أكثر امراض التهاب الأوعية الدموية ألتي تصيب الجلد و المفاصل و الجهاز الهضمي و الكليتين لدى الاطفال. أعد هذا البحث للاطلاع على الجوانب السريرية و الوبائية لفرفرية هينوك–شونلاين لدى الأطفال الكرد في مدينة دهوك.

طرق البحث: تمت دراسة الأطفال المصابين بفرفرية هينوك–شونلاين،الذين عولجوا في مستشفى هيفي التعليمي للفترة من شهر تموز 2009 و لغاية تموز 2011 من حيث العمر و الجنس و العلامات الأولية والعلامات الأخرى للمرض، و العوامل المؤهبة و الفترة التي ظهر فيها المرض.الفحوصات المختبرية التي أجريت شملت فحص الدم العام و معدل ترسيب الكريات الحمر و وظائف الكلية وفحص الإدرار العام, وأخذت خزعة من الجلد في احد عشر مريضا. تمت متابعة المرضى لفترة ستة اشهر بأجراء فحص الادرار العام ووظائف الكلية لتسجيل حالات اصابة الكلية.

النتائج: تم تشخيص 51 مريضا" خلال سنتين، منهم 28 ذكرا و 23 أنثى. تراوحت أعمار المرضى بين عشرة اشهر و خمسةعشر سنة، و معدل عمر المرضى كان 7.1سنة و نسبة الذكور إلى الإناث كان 1:1.2 . معظم المرضى تم تشخيصهم في فصلي الشتاء والخريف. جميع المرضى كان لديهم فرفرية الجلد. التهاب او آلام المفاصل الكبيرة سجل في 41 مريضا"(80.4%)، آلام البطن في 24 (47%) مريضا" و إصابة الكلية في حالة واحدة. حصل شفاء تام في جميع المرضى. بعد ستة اشهر من متابعة المرضى لم تسجل اية اصابة في الكلية.

الاستنتاجات: : الخصائص الوبائية والاحصائية لفرفرية هينوك–شونلاين في الاطفال الكرد تشبه ماهو موجود في باقي الدول ولكنها تتميز بانها اقل شدة مع اصابات قليلة جدا في الكلية.

CROHN'S DISEASE IN AN INFANT PRESENTED WITH PERFORATION OF THE COLON: A CASE REPORT

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SUMMARY

Infantile Crohn's disease is very rare and has been described as a severe illness with poor prognosis. We report the clinical, hematological, radiological and pathological findings of an 11 month-old boy with infantile colonic Crohn's disease who presented with repeated vomiting, fever and pallor. Chest X ray showed an air under the diaphragm. On laparotomy there was perforation of the colon. Although rare, Crohn's disease should be considered in the differential diagnosis of any child with sever gastrointestinal symptoms.

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Key words: Chronic inflammatory bowel disease, Crohn's disease, Infancy, Intestinal perforation

Crohn's disease, an idiopathic chronic inflammatory disorder of the bowel, involves any region of the alimentary tract from the mouth to the anus. About 70% of the patients has terminal ileitis alone and in 10%-15% there is only colonic involvement.[1] All age groups are affected but it is most commonly diagnosed between the ages of 15-35. It rarely occurs in children below 10 years and it is extremely rare in infants less than 1 year of age.[2] We report an 11 month-old infant who presented with intestinal perforation due to Crohn's disease.

CASE PRESENTATION

An 11-month-old boy was admitted to the emergency department at Heevi Pediatric Teaching Hospital in Duhok governorate suffering from repeated vomiting and fever. On physical examination the child was dehydrated and pale and had rigid abdomen. Complete blood test was done which revealed a hemoglobin concentration of 5 gm/dl and total white blood cell count of 37,600/mm.[3] Plain abdominal x ray showed air under the diaphragm (Figure 1). The patient received blood transfusion. Written informed consent was obtained from the patient's guardian. Then exploratory laparotomy was done which showed multiple colonic perforations. The bowel walls were inflamed and matted together by thin fibrinous adhesion. Colostomy done at the site of perforation and biopsy was taken. Postoperatively, the patient started to regain his bowel function, became better generally well and started oral feeding. At the third postoperative day, the general conditions of the patient deteriorated, the colostomy stopped functioning and the site of drain started discharging fecal materials. Another exploratory laparotomy was done which showed multiple perforations at the left descending and sigmoid colon distal to site of previous colostomy. Peritoneal irrigation was performed followed by resection of the

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perforated segment of the colon. The removed part of the colon was sent for histopathological investigation. Grossly, the segment showed mucosal surface with multiple ulcerations and normal skipping areas giving rise to a cobblestone appearances. The wall is thickened with adhesion of creeping of pericolic fat (Figure 2). Microscopically sections of the large bowel wall showed variable depth ulceration, necrosis with chronic inflammatory cells (plasma cells, lymphocytes, histocytes and few giant cells) and non-caseating vague granlomas near the blood vessels with pericolitis (Figure 3 and 4).

DISCUSSION

Crohn's disease has rarely been observed in infancy.[3] Between 1990 and 1998, three siblings and their first degree cousin were found to have Crohn's disease and their respective ages on diagnosis were 3 weeks, 2 weeks, 3 months and 2 months.[4] In these cases, the inflammatory process involved extensive anorectal and colon involvement with sparing of the small bowel. There were sever perianal disease consisting of ulceration, mucosal and skin tags, fistulae and fissures. In our patient, there was also involvement of colon with sparing of small bowel. The main presentation of our patient was chronic constipation followed by intestinal perforation. The first known infant affected by Crohn's disease was reported by Koop et al in 1947.[5] Miller and Larson[6] reported 12 cases of Crohn's disease in the newborn period. In 8 of the cases reviewed, the disease involved the small bowel, whereas in 3 the disease involved the right colon and terminal ileum; one patient had only colonic involvement. Seven of 12 patients reviewed by Miller and Larson died after surgery. The patients reported by Koop et al and Killer and Larson had a prodromal period of diarrhea lasting from 2 days to several weeks, followed by signs of intestinal obstruction. None of these had any perianal involvement. Mezot et al[7] reported a 4-week-old infant with signs and symptoms suggesting Crohn's disease that initially associated with central nervous system thrombosis and later, at the age of 6 months, with sever perianal inflammations. In conclusion, although rare, Crohn's disease in infancy should be considered in the differential diagnosis of any infant with sever chronic gastrointestinal manifestation.

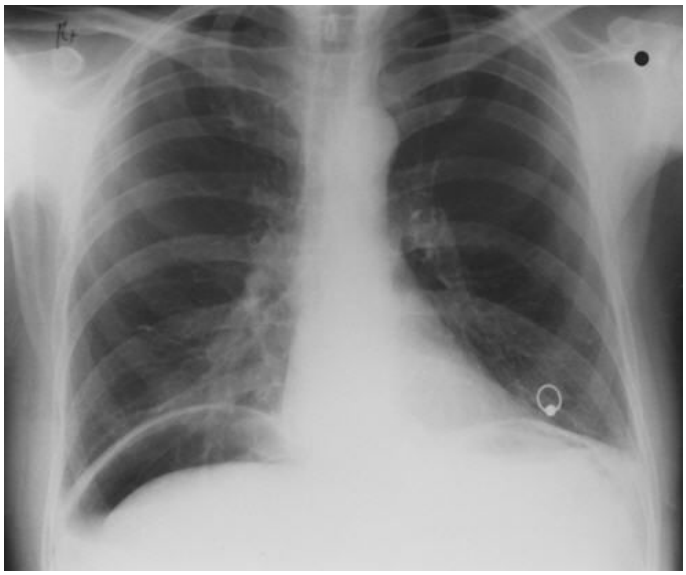

Figure 1. Chest X ray shows air under the diaphragm

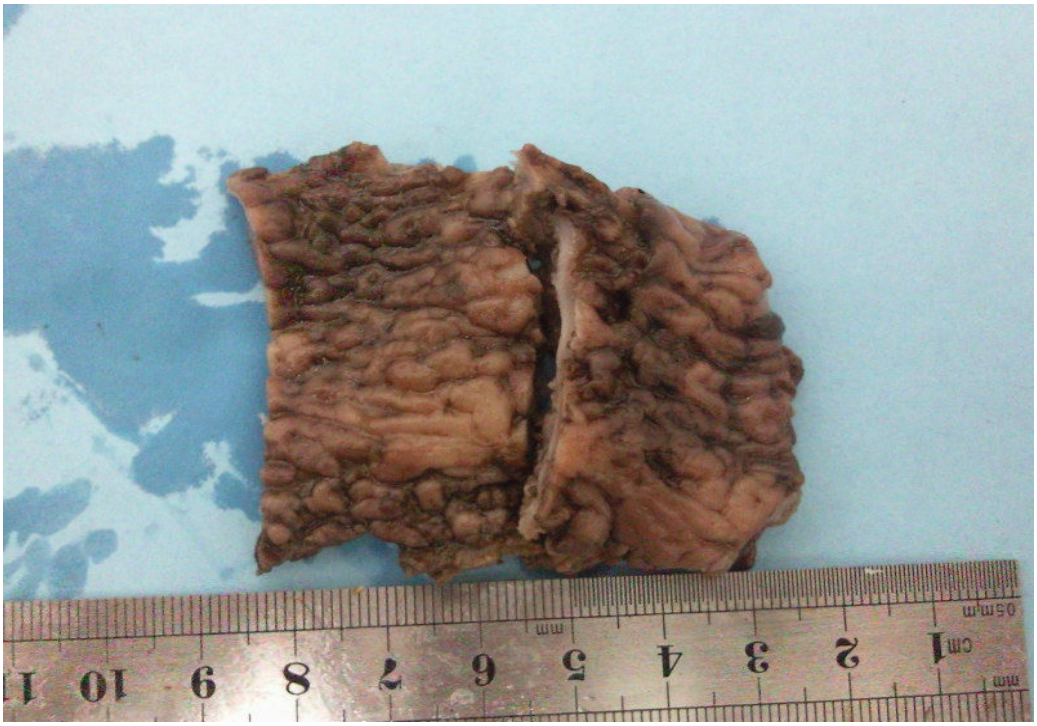

Figure 2. Large bowel segment reveal cobblestone appearances with presence of skipping lesion

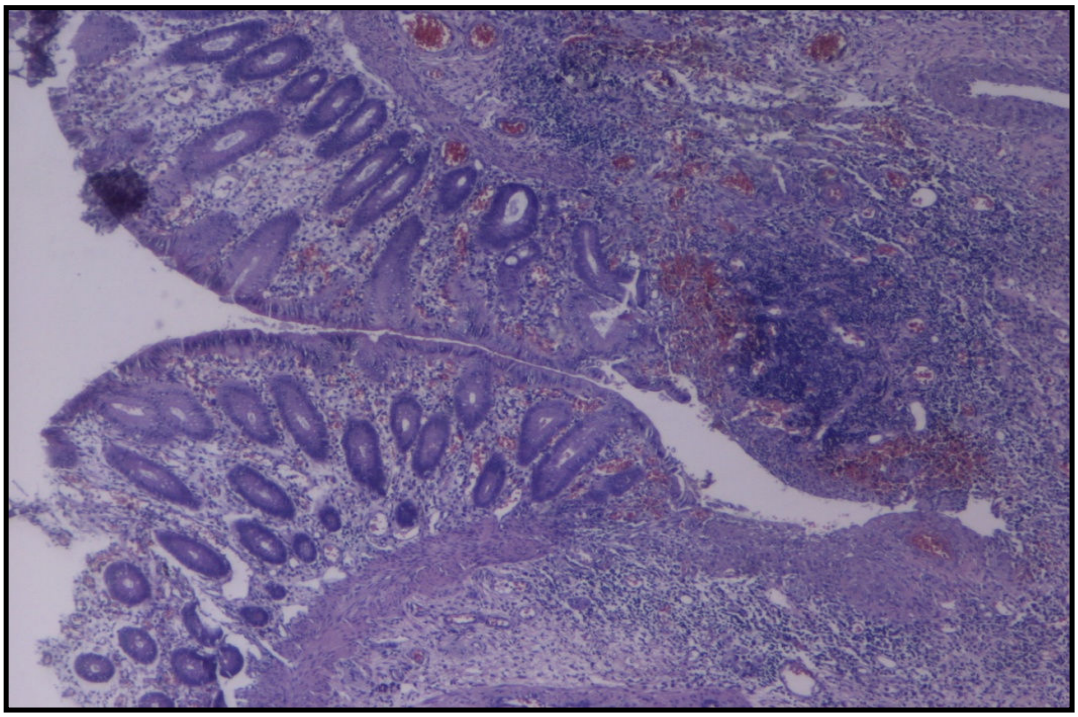

Figure 3. Ulcer that reach the sub mucosa with vague granuloma at the base and normal edematous mucosa at edges of the ulcer

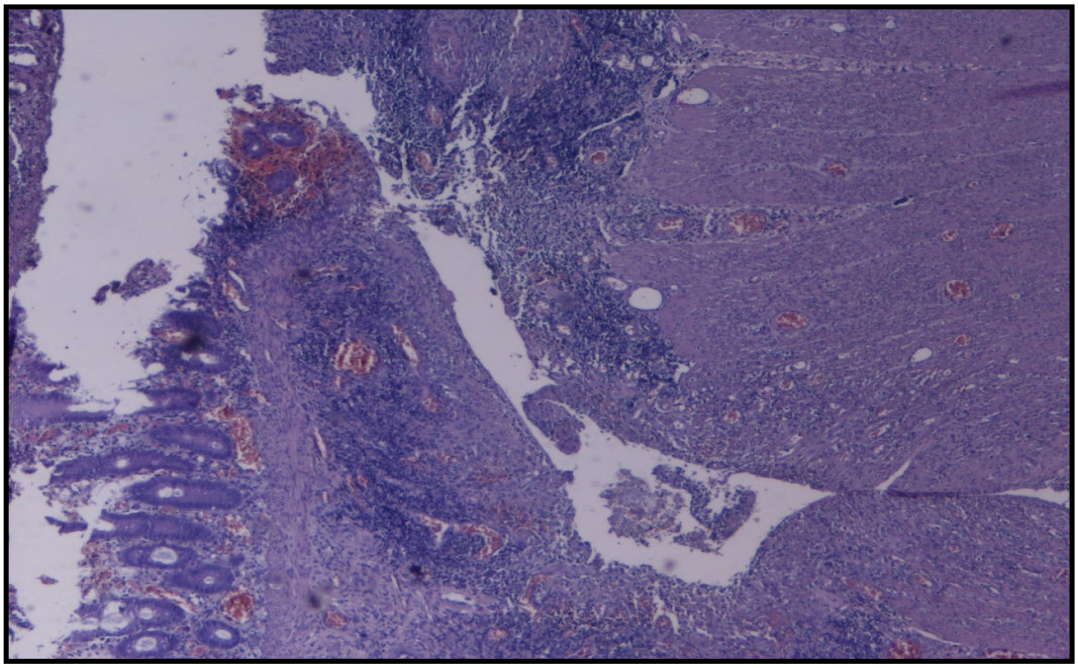

Figure 4. Fissure throught the muscularis propria

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پوختە

ئێشا كرۆنی ل زاروكی دگەل هەبونا كونی ل قولونێ (ریڤیكا ستویر): دۆزا تومارێ

نساخییا كرونێ ل سافایان زورا دەگمەنە و هاتیە راڤەكرن وەك ئێشەكا گران و بەربینیەكا كێم. مە تومارکرب رێكا دیتنت كلین و هیماتولوجی (بكار ئینانا خینێ) وتیشكی وبایوپسی (وەرگرتنا نمونەی ل لەشی و تاقیكرنا وی) ل زاروكەكی كور 11 هەیفی كو دنالی ب سەدەما نساخییا كرونی یا قولونێ كو پێڤە دیاربو ڤەرەشیانەكا بەردەوام و تا و زەرایی. ب تیشكا X یا سینگی هاتە دیاركرن كو هەوا ل بن پەردەیا ناڤبرێ دا هەیە و دڤەكرنا زكی دا كونبونا قولونێ هەبو هەرچەند یا كێم بوو. ئەڤ نساخیە دفێت بهێتە بەر چاڤ كرن ل هەر زاروكەكی ئەوێن دنالێن ب سەدەما نیشانێن ئێشێن ئورك و ریڤیكان.

الخلاصة

داء الكرون في الاطفال يتمثل بوجود ثقب في القولون: تقريرالحالة

مرض الكرون في الاطفال نادر الحدوث ويوصف بشدة المرض وصعوبة تشخيصة. حيث ياخذ الحالة السريرية واجراء فحوصات الدم والأشعة واخذ عينة من القولون للأطفال التي اعمارهم 11شهر والمصابون بهذا المرض والذي لديهم التقيوء والحمى والشحوب، عند اجراء اشعة الصدر يلاحظ وجود الهواء تحت الحجاب الحاجز وجراحيا يلاحظ ثقب في القولون مع انه نادر الحدوث. مرض يجب أن يؤخذ بنظر الإعتبار في التشخيصِ التفاضلي لأيّ طفل بالأعراض المعوية الحادّة.

AMIODARONE- INDUCED THYROIDITIS IN A POST-CARDIAC TRANSPLANT PATIENT – CASE REPORT

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SUMMARY

Amiodarone is a class III antiarrhythmic medication that is widely used for the treatment of various arrhythmias and is useful in non-ischaemic dilated cardiomyopathy. It is well known that amiodarone can alter the biochemical status of the thyroid gland. Amiodarone-induced thyroidits is more common than amiodarone-induced hypothyroidism. It is estimated that 23% of patients receiving amiodarone may develop amiodarone-induced thyroidits. We are reporting a case of a young lady with familial dilated cardiomyopathy who was on amiodarone that was stopped in July 2007, yet she developed clinical signs and biochemical evidence of amiodarone-induced thyroidits few months after her cardiac transplantation despite stopping amiodarone. She was managed conservatively jointly with the endocrinologist and her thyroid function test returned back to normal eventually.

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Key words: Amiodarone, Thyroiditis, Dilated cardiomyopathy, Cardiac transplant

Amiodarone is an effective antiarrhythmic medication that is used in treatment of various arrhythmias and it is also useful in non-ischemic dialted cardiomyopathy. It is a benzofuran-derived, iodine-rich drug with many structural similarity to thyroxine (T4). Amiodarone contains around 37% iodine by weight. Every 200-mg tablet is estimated to have about 75 mg of organic iodide, 8-17% of which is released as free iodide. Standard maintenance treatment with 200 mg amiodarone can provide more than 100 times the usual daily iodine requirement. It is highly lipid-soluble and is concentrated in the adipose tissue, lung, muscle, liver, and thyroid gland. It may cause severe side effects, including thyroiditis; hypothyroidism and thyrotoxicosis. This may present even after discontinuation of the drug as the the elimination half-life of amiodarone is variable, ranging from 50-100 days; total body iodine stores remain increased for up to 9 months after discontinuation of the drug.

CASE PRESENTATION

A 20-year old Omani lady who has been diagnosed to have "familial dilated cardiomyopathy" complicated with congestive heart failure for three years with intracardiac defibrillator (ICD) being inserted in 2005. She was on warfarin, spironlactone, frusemide, valsartan, carvidelol, amiodarone and ranitidine. She was first admitted in Sultan Qaboos University Hospital (SQUH) in January 2007 with community acquired pneuomonia (CAP) complicated by decompensated heart failure status. She was found to have BP of 80/50 mmHg, tachycardia of 110 beats per minute,

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displaced apex, mitral regurgitation (MR) and tricuspid regurgitation (TR) murmurs, congestive hepatomegaly and lower limbs edema. The laboratory investigations on admission were as shown in table 1.

Table 1. Baseline investigations as on first admission

Test	Result
Haemoglobin	13.9 g/dl
WCC	5.8 x 10^9/L
Neutrophils	3.0 x 10^9/L
Platelet	187000 /L
Serum sodium	128 mmol/L
Serum potassium	4.1 mmol/L
Serum chloride	97 mmol/L
Serum bicarbonate	23 mmol/L
Serum urea	6.1 mmol/L
Serum creatinine	58 umo/L
eGFR	> 90 ml/min/1.73 m^2

Her chest X-ray showed cardiomegaly and pulmonary congestion along with lobar pneumonia in right lower lobe. Her echocardiography (ECHO) showed grossly dilated 4 heart chambers with severe global hypokinesia. Her LVEF was 28% and moderate MR and TR. Her thyroid function tests (TFT) on admission were within the reference ranges.

She was treated with anti-heart failure medications and antibiotics, she made good recovery from her CAP and her heart failure was reasonably controlled She was discharged on 07/07/2007 in a stable condition with a follow up in cardiology clinic. Her medications included all the drugs mentioned above apart from amiodarione which was stopped.

The patient underwent cardiac transplantation in China in August 2007. In her follow up at SQUH in November 2007 she was asymptomatic and free from signs of cardiac failure. However, she was found to have diffusely enlarged goiter with no tenderness and no overlying bruit or local lymphadenopathy. Nevertheless, her laboratory investigations including full blood count, renal and liver functions were all within the references ranges.

Her repeated ECHO showed mild left ventricular hypertrophy with mild hypokinesia of the anterior wall. Her LVEF was 60% with only trivial MR and TR. Post transplantation, she was kept on captopril 25 mg TID, sirolimus 2 mg OD, mycophenolate 750 mg BiD and carvedilol 12.5 mg BD. Her TFT showed TSH 0.02 mU/L, FT3: 8 pmol/L and FT4 27 pmol/L, pattern of low TSH with slightly raised FT3 and FT4. Thyroid antibodies were negative. Thyroid uptake scan was done and showed global low uptake of 0.1% suggestive of thyroiditis (Figure 1). However, the patient remained clinically euthyroid. She was reviewed by endocrinologist who advised close monitoring and not to start any anti-thyroid medications like thioamides. The patient had serial TFTs which returned back to normal ranges in July 2008 and she maintained these values to date (Table 2). The patient was euthyroid throughout this period.

Table 2. Thyroid function tests on admission and during follow up

TFT	6/11/2007	15/1/2008	29/7/2008	6/1/2009	7/7/2009
FT3 (3.8-6 pmol/l)	7.8	9.5	5.1	5.1	4.6
FT4 (7.9-14.4 pmol)	18.2	27.0	8.1	9.7	8.1
TSH (0.34-5.6 mIU/l)	0.02	0.02	1.51	1.31	1.08

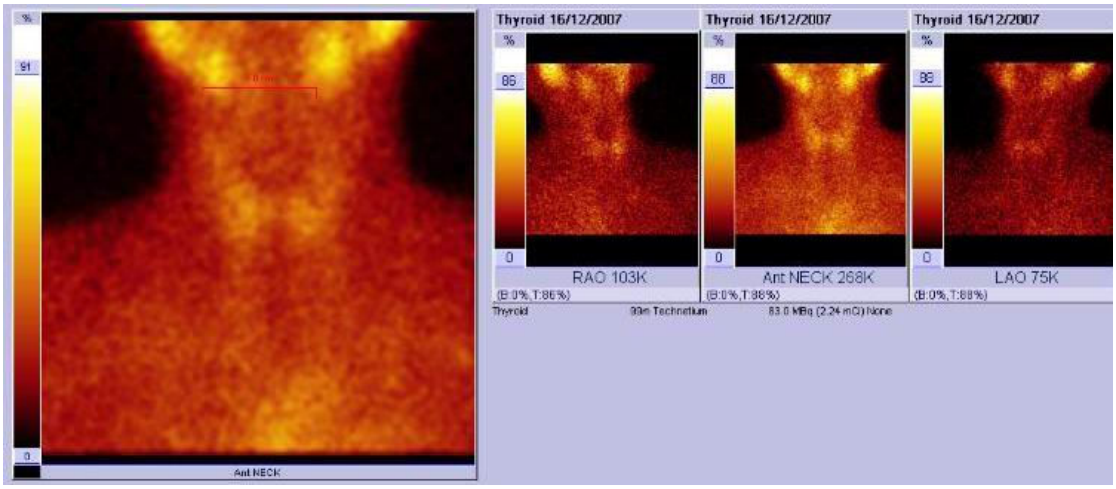

Figure 1. Thyroid uptake scan showing low uptake of 0.1%

DISCUSSION

Amiodarone is a class III antiarrhythmic medication that is widely used in the treatment of various arrhythmias and is useful in non-ischaemic dilated cardiomyopathy (NIDCM).[1] It is well known that amiodarone can alter the biochemical status of the thyroid gland. It is estimated that 23% of patients receiving amiodarone develop amiodarone-induced thyroidits (AIT).[2]

Amiodarone induced thyroidits can be classified into two types according to their mechanism. In type 1 which is the latent disorder, there is synthesis and release of excessive thyroid hormones whereas in type 2 which is more common, there is increase in the release of thyroid hormones due to the destructive effect of amiodarone on the thyroid gland.[2] Thyroid antibodies, iodine uptake scan and doppler studies can be used to differentiate the two forms. Combination of both types was reported in some patients and differentiation of the two types is not always easy.[2]

Interestingly, AIT can present despite discontinuation of amiodarone therapy. Patients who were on amiodarone before heart transplantation are prone to AIT for a year after discontinuing amiodarone. Careful monitoring of thyroid function is recommended for those patients.[3]

Patients with AIT are known to be prone to major adverse cardiovascular event (MACE). In a study done in Hong Kong enrolling 354 patients defined MACE as cardiovascular mortality, myocardial infarction, stroke and heart failure, or ventricular arrhythmias that required hospitalization. They concluded that AIT is associated with a 2.7-fold increased risk of MACE.[4]

Although amiodarone-associated thyroid dysfunction is usually a mild clinical condition that can subside spontaneously in approximately 20% of patients.[5] However, it can be severe, life threatening, and even fatal. However, the long term prognosis for AIT is usually good. [6]

In conclusion, Amiodarone-associated thyroid dysfunction can present despite discontinuation of amiodarone therapy as this drug has a very long elimination half life and this is more in those with slow acylators. It is important for physicians who regularly prescribe amiodarone to be aware of its consequences on the thyroid function especially in long-term users. Thyroid hormone levels and antibodies should be done as a baseline and repeated every six months or earlier if clinical features of thyroid dysfunction appear in patients on admiodarone.

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پوختە

نەخوشیا تایرودایتس ژ ئەگەرێ ب کارئینانا ئەمایوداروونی پشتی نەخوشیا چاندنا دلی – راپورتکرنا حالەتەکێ

ئەمایوداروون ئیك ژ دەرمانێن د هیتە ب کارئینانا بو ڕێکخستنا لێدانا دلی هەروەسا بو نەخوشیا (Non-ischemic dilated cardiomyopathy). و دهیتە پێشبینیکرن کو 23٪ ژ نەخوشێن ئەڤی دەرمانی ب کاربیین توشی هەودانا تایرودی بن. ئەڤە راپورتا نەخوشەکا ئافرەتە کو نەخوشیا (Familial dilated cardiomyopathy) کو ل تەممووزا 2007 ئەڤ دەرمانە ب کار نە ئینای بەلێ هەر تووشی هەودانا تایرودی بوی چەند هەیڤا پشتی نشتەرگەریا چاندنا دلی. ئەڤ نەخوشە هاتە چارەسەرکرن ب ڕێکێن ئاسای وەك ب کارئینانا ئەنتی بایوتیکا و دەرمانێن دژی وەستاندنا دلی و تاقیکرنا کارێ تایرودی هاتە زڤراندن بو حالەتێ نورمال.

الخلاصة

التهاب الغدة الدرقية نتيجة استعمال الامايودارون بعد اجراء عملية زرع القلب – اشهار حالة

الامايودارون هو أحد الادوية التي تستعمل في علاج عدم انتظام ضربات القلب كما هو مفيد في علاج مرض (Non-ischemic dilated cardiomyopathy). و من المعروف بأن هذا الدواء يودي الى خلل في وظائف الغدة الدرقية. و يذكر بأن التهاب الغدة الدرقية نتيجة استعمال هذا الدواء هو أكثر شيوعا من نقص هورمون الغدة الدرقية نتيجة استعمال الدواء. و قد قمنا برصد حالة شابة مصابة ب (Familial dilated cardiomyopathy) و التي كانت تستعمل الدواء الى حين توقف الدواء في تموز 2007. رغم عدم استعمال الدواء فقد ظهرت لدى المريضة العلامات السريرية و البايوكيمياوية لالتهاب الغدة الدرقية نتيجة استعمال هذا الدواء عدة أشهر بعد اجراء عملية زرع القلب. تم علاج المريضة تحفظيا حيث تم ارجاع وظائف الغدة الدرقية الى حالتها الطبيعية.