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Iraqi Journal of Medical Sciences publishes original articles, case reports, and letters to the editor, editorials, investigative medicine, and review articles.

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Types of Contributions: Original articles, review articles, case studies, editorials, medical education, history of medicine, ethics, practical points, medical quiz, conferences, meetings and letters to the Editor.

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3. Chapter in book: Phillips SJ, Whisnant JP. Hypertension and strock. In: Laragh JH, Brenner BM. editors. Hypertension: Pathophysiology, diagnosis, and management. 2nd ed. NewYork: Raven Press; 1995. p. 465-78.

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Conflict of Interest

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Abstract

Conflict of interest (COI) in peer review journals is a serious issue that earn more attention than it currently receives. COI can influence reviewers' or editors' decisions in favor of or against a project. There is no universal description of COI in the medical and scientific world; it is generally defined as a set of situations, in which a major professional interest is overly influenced by a secondary one(s) authors, editors, and reviewers may all be subject to COI. The majority of COI discussion in scientific papers has centered on the impact that authors' financial and nonfinancial (i.e., personal or professional) circumstances have the impact that personal (or professional) interests can have on research integrity.

Keywords Conflict of interest, authors, editors, reviewers, budget

Citation Hassan JS. Conflict of interest. Iraqi JMS. 2022; 20(1): 1-2. doi: 10.22578/IJMS.20.1.1

Introduction

When you send a research to a scientific journal or when a scientific journal sends you a research for the purpose of evaluation, the journal will ask you for the term conflict of interest (COI), what is it and why is it requested.

An interest in general is something that has value or influence on a person or group of individuals [(1)]. So, a COI in life arises when the goals or interests do not coincide between two people or a group of people or even between more than one institution, but the pursuit of interests is one and within different approaches [(2)]. Therefore, it is logical that a COI is the situation, in which a compromise must be reached; it is immoral to think that COI is a personal matter, but rather the result of pursuing goals in different ways, so is not something more dire [(3)].

Conflict of interest in research

In scientific research, COI occurs when the researcher, reviewer, or editor-in-chief has a special interest that may affect the fairness or harm his impartiality, or there is a COI in the research itself that may lead to questioning its integrity [(4)].

Conflict of interest to reviewer

As a reviewer, you may find out that the study you're reviewing is very similar to, or even competes with, the paper you're now creating, lowering the relevance of your research. You might dismiss it as insufficient in that instance. It is here that a research conflict of interest arises [(5)].

Types of conflict of interest

There are majorly four types of COI as listed below:

- Budget
- Personal

- Contractual
- Professional

When it comes to this topic, it must be pointed out to the personal or professional relationships. For example, when you were with the researcher or writer in the same institution in the past or even in the present. Likewise, the author could be a close friend of yours. There is COI in each of the examples cited [6].

Budget Conflict of interest

Financial links, including direct COI such as employment, stock ownership, grants, and so on, are the most typical COI. This form of dispute involves an individual's financial reward that can help him or her in the future [5].

Point to consider

One of the requirements for publishing article or to became a reviewer that researchers and reviewers face throughout the world is COI. Reporting these conflicts to the institution's ethical group as soon as possible is the best way to deal with them. The editorial decision to publish the research is based on the disclosure of COI. Many researchers or reviewers neglect the ability COI, thought that it will not strike their decision. The correct solution in such a case is to authorize and write to the editorial board and leave the choice for them to make the decision

Corresponding to the U.S office of research integrity [3]. "Having a COI is not in itself unethical, and some are unavoidable. Full transparency is always the best course of action, and, if in doubt, disclose."

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The Potential Role of Epstein-Barr Virus Nuclear Antigen-1 (EBNA-1) in Multiple Sclerosis

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Abstract

Background Multiple sclerosis (MS) is a disease affecting the central nervous system (CNS), with inflammation and demyelination of nerves, eventually resulting in nerve damage and disabilities. The risk of developing MS enlarged by infectious mononucleosis, which is caused by delayed primary infection by Epstein-Barr Virus (EBV). Early reports consistently demonstrated an increased antibody response in MS patients versus healthy subjects towards different EBV antigens, including the EBV nuclear antigen-1 (EBNA-1). In Iraq, number of researchers found that the EBV have a possible role at some point in the course of the disease, others reported that the EBV pathogenesis have an important role in the triggering of MS disease.

Objective To compare the sero-prevalence of EBV nuclear antigen-1 antibody (EBNA-1 IgG) among the Iraqi MS patients and controls and find out whether there is a relation between disease severity and EBNA-1 IgG titer.

Methods This case-control study conducted on 120 MS patients aged between 13-42 years, and 120 apparently healthy age- and sex-matched volunteers as controls. Three ml of whole blood were collected from all MS patients and controls and put in gel tubes. The blood samples were centrifuged at 5000 RPM for 5 minutes to get serum from the gel tubes. Serum was preserved in (-20°C), and then used for enzyme-linked immunosorbent assay (ELISA). The ELISA study was performed using ELISAs kits (Abnova /Taiwan) for EBNA-1 IgG antibodies measurement.

Results EBNA-1 IgG was positive in 51.7% (62/120) of MS patients and 39.2% (47/120) of controls, (P=0.035). The median of EBNA-1 IgG level of MS patients and controls were 81.08 U/ml, and 67.73 U/ml, respectively (P=0.043). And EBNA-1IgG was significantly higher in younger age groups. Patients with the first-line and second-line treatment showed no significant differences in EBNA-1 IgG levels, while the median level in patients without treatment (newly diagnosed) and those who were at early years of the disease was higher.

Conclusion EBNA-1 antibody could play a triggering role in MS because it is significantly higher in MS patients than in controls, especially at younger age groups, early stages of the disease and in female patients.

Keywords Multiple sclerosis, EBV, EBNA-1 IgG, ELISA

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List of abbreviations: EBV = Epstein-Barr virus, EBNA-1 IgG = Epstein-Barr virus nuclear antigen-1 antibody, ELISA = Enzyme-linked immunosorbent assay, IgG = Immunoglobulin G, MRI = Magnetic resonance imaging, TMB = Tetramethylbenzene

Introduction

Multiple sclerosis (MS) is a disease affecting the central nervous system (CNS), with inflammation and demyelination of nerves, eventually resulting in

nerve damage and disabilities [1]. Young adults are most commonly affected by MS while women are more susceptible and men have worse progression [1,2]. It is assumed that both genetic and environments factors are effective on this disease [3].

Epstein-Barr virus (EBV) is also referred to as the human gamma herpesvirus, the disease is asymptomatic in childhood, but it becomes symptomatic in adolescents. The frequency of MS is nearly 15 times higher in early childhood with EBV and about 30 times higher among adolescent and later-life patients who have EBV infections [4]. An increased antibody response has been seen in MS patients versus healthy subjects towards different EBV antigens, including the EBV nuclear antigen-1 (EBNA-1) [5].

EBNA-1 is expressed in all actively dividing EBV-infected cells and is responsible for fusion of the viral episome to the mitotic cellular DNA, confirming duplication and transport of virus genome to all daughter cells [6]. EBNA-1, the essential EBV antigen for virus latency, makes up a principal antigen for both cell-mediated and humoral immune responses against the virus, and in MS the deregulation of immunity specific for EBV has been reported principally for this antigen [7]. Increased levels of EBNA-1-specific antibody responses may also predict conversion from clinically isolated syndrome (CIS) to MS [8].

Antibody responses to EBNA-1 reach their highest titer during convalescence from infectious mononucleosis. Notably, epidemiological studies reported that the increase in EBNA1-specific IgG titers precede the onset of MS in various populations [9]. Ascherio et al. [9] found that IgG specific for EBV nuclear antigens were significantly elevated in plasma collected before the onset of MS and did not change significantly after MS onset.

To the best of our knowledge, there are two previous studies regarding the seroprevalence of EBNA-1 IgG in Iraq, Bakir et al. in 2017 showed significantly higher level in MS patients than the healthy controls and he was revealed the possible role of EBV during the course of MS autoimmune disease via antibody EBNA-1 IgG seropositivity and correlated with type of attack or relapse [10], Abd Al Kareem and Abd in 2020 reported that mean serum level was significantly higher in female patients than in the healthy controls and they revealed that the pathogenesis of EBV has crucial role in initiation MS disease in females patients [11]. The objective of this study is to find out any correlation between MS development and the incidence and levels of anti- EBNA-1 IgG.

Methods

A case-control study conducted on 120 patients with MS aged between 13-42 years from November 2020 to June 2021. Blood samples were obtained from MS patients in the MS clinic in the Baghdad Teaching Hospital of Medical City Complex, and 120 controls were obviously healthy age and sex-matched volunteers collected from the blood donation centers. Informed consents were obtained from all subject before sampling. This study was approved by the Institutional Review Board of the College of Medicine, Al-Nahrain University (No.20200980 on 17/11/2020). The study was conducted in the labs of Microbiology Department at the College of Medicine -Al-Nahrain University. Patients' clinical parameters included: the duration of MS, number of relapses, the line of treatment and type of treatment. Exclusion criteria: Patients on rituximab therapy. Three ml of whole blood were collected from all MS patients and controls and put in gel tubes. The blood samples were centrifuged at 5000 RPM for 5 minutes to get serum from the gel tubes. serum was preserved in (-20^{o}C), and then used for enzyme-linked immunosorbent assay (ELISA).

The ELISA study was performed using ELISAs kits (Abnova/Taiwan) for EBNA-1 IgG antibodies measurement depended on the binding of antibodies in the sample with EBNA-1 antigen that coat the wells of ELISA plate and the antibodies being in complexes with antigen are later recognized by animal anti-human IgG

antibodies labelled with horseradish peroxidase. The labelled antibodies are revealed by an enzymatic reaction with a chromogenic substrate. Sensitivity of the test was 100%, and Specificity was 96.4%.

For quantitative evaluation the sample antibody titers in artificial units (AU/mL) were computed as follows: 1. A calibration curve was constructed by plotting the units of Standards (x-axis) to absorbance of Standard (y-axis). 2. The place where the absorbance of tested samples intersect calibration curve were found and the corresponding values (AU/mL) on the axis x were found.

Statistical Analysis

Statistical package for social sciences (SPSS) Inc., Chicago,IL, USA, Version 21 was used for statistical analysis; categorical data were formulated as count and percentages, and Chi-square test was used to describe the association of these data. Numerical data were described as the median with percentile. And independent sample t-test was used for comparison between the two groups. The lower level of statistically significant difference was regarded as ≤ 0.05.

Results

Among the 120 MS patients; 48 (40%) were males and 72 (60%) were females, there was no significant difference in sex distribution between patients and controls (P= 0.447). The median age of MS patients was 32 years and also there was no statistically significant difference between the age of the MS patients and controls for the different age groups indicating that they were of a comparable age (P= 0.465). According to the duration of MS disease in the studied patients; 75% of MS patients (90 out of 120 patients) had MS for more than 2 years, most of MS patients (76.67%) 92 out of 120 were having a low number of relapses of less than 3 relapses, and the lower percentage of patients (23.33%) 28 out of 120 were having a higher number of relapses of more than 3 relapses.

The treatments used by MS patients included Avonex (β-interferon-1a), Betaferon (β-interferon-1b), Rebif (β-interferon-1a), Gilenya (Fingolimod), Natalizumab (Tysabri), and the last group were without treatment, most of them were newly diagnosed. The largest group of the patients were on Natalizumab regimen (40.83%), and the smallest group of the patients were on Avonex (5%). Large number of MS patients (48.33%) treated with the second line therapy that included (Natalizumab or Gilenya).

The results of ELISA showed that EBNA-1 IgG was positive in 51.7% (62/120) of MS patients and 39.2% (47/120) of controls, The median of EBNA-1 IgG level of MS patients were 81.08 U/ml (Percentile 25=57.88, Percentile 75=101.40), and of control were 67.73 U/ml (Percentile 25=54.62, Percentile 75=103.93), (Table 1).

Table 1. Comparison of EBNA-1 IgG index values between MS patients and controls

EBNA-1 IgG	MS		Control		P-value
Negative	58	48.3%	73	60.8%	0.035*
Positive	62	51.7%	47	39.2%	
Median	81.08		67.73		
Percentile 25	57.88		54.62		0.043*
Percentile 75	101.40		103.93		

This study showed that the rate of seropositivity and median of EBNA-1 IgG level of the MS patients were significantly higher than the controls in relation to the age groups:

(<21 years) ((P= 0.050, 0.006), and (21-30) (P= 0.003, <0.001) respectively; whereas in the remaining age groups (31-40), (>40), there was no significant differences in the median level and seropositivity rate of EBNA-1 IgG between MS patients and controls, (Table 2).

Table 2. EBNA-1 IgG results in MS patients and controls in relation to age groups

Age range	EBNA-1 IgG	MS		Control		P value
<21 years	Negative	6	42.9%	10	83.3%	0.050*
	Positive	8	57.1%	2	16.7%	
	Median	80.85		54.42		
	Percentile 25	55.23		45.73		0.006*
	Percentile 75	94.58		63.84		
21-30 years	Negative	20	42.6%	30	75.0%	0.003*
	Positive	27	57.4%	10	25.0%	
	Median	94.83		63.59		
	Percentile 25	55.63		54.92		<0.001**
	Percentile 75	102.32		79.94		
31-40 years	Negative	27	55.1%	30	49.2%	0.569
	Positive	22	44.9%	31	50.8%	
	Median	73.62		80.62		
	Percentile 25	59.32		56.24		0.755
	Percentile 75	104.99		112.46		
>40 years	Negative	5	50.0%	3	42.9%	0.995
	Positive	5	50.0%	4	57.1%	
	Median	86.66		81.31		
	Percentile 25	61.81		59.53		0.894
	Percentile 75	99.43		126.14		

**P value highly significant <0.001, *P value is significant <0.05

The median of EBNA-1 IgG titer was significantly higher in females compared to the males (P= 0.004) (Table 3), in addition, females have higher percentage of IgG seropositivity than males (59.5 % in females versus 39.1% in males), as shown in the table (3).

Table 3. ENBA-1 IgG result in relation to sex among MS patients and controls

EBNA-1 IgG	Study groups			
	MS		Control	
	Female	Male	Female	Male
Negative	30 (40.5%)	28 (60.9%)	44 (61.1%)	29 (60.4%)
Positive	44 (59.5%)	18 (39.1%)	28 (38.9%)	19 (39.6%)
Median	94.58	63.91	66.67	67.84
Percentile 25	64.12	53.81	53.40	55.84
Percentile 75	104.99	97.88	109.79	101.79
P value	0.004*		0.995	

The rate of seropositivity and median of EBNA-1 IgG is significantly higher in the patients who have the disease for 2 years or less than in the patients with a disease duration more than 2 years (p<0.001, P=0.029 respectively), (Table 4).

Table 4. The relation between EBNA-1 IgG serology and disease duration

EBNA-1 IgG	Disease duration		P value
	≤2 years	>2 years	
Negative	5 (16.67%)	53 (58.89%)	<0.001**
Positive	25 (83.33%)	37 (41.11%)	
Median	82.81	64.22	
Percentile 25	55.43	53.81	0.029*
Percentile 75	126.14	97.37	

**P value highly significant <0.001, *P value is significant <0.05

This study observed no statistically significant association of EBNA-1 IgG serology result with number of relapses (p= 0.812), as shown in table (5).

Results in table (6) illustrated that there was no statistically significant association of the EBNA-1 IgG results with line of treatment (p=0.549).

Table 5. The relation between EBNA-1 IgG serology and number of relapses

EBNA-1 IgG	Number of relapses		P value
	≤3 relapses	>3 relapses	
Negative	45 (48.91%)	13 (46.43%)	0.812
Positive	47 (51.09%)	15 (53.57%)	
Median	68.38	64.86	
Percentile 25	55.02	54.21	0.833
Percentile 75	107.59	96.56	

**P value highly significant <0.001, *P value is significant <0.05

Table 6. The relation between EBNA-1 IgG serology and line of treatment

EBNA-1 IgG	Line of treatment			P value
	No treatment	1st line	2nd line	
Negative	5 (41.67%)	22 (44.00%)	31 (53.45%)	0.549
Positive	7 (58.33%)	28 (56.00%)	27 (46.55%)	
Median	94.87	63.59	66.67	
Percentile 25	64.17	54.42	53.2	0.390
Percentile 75	144.13	101.26	102.32	

Discussion

MS is a CNS disease characterized by demyelination, inflammation, and neuronal destruction. Genetic and environmental factors are coupled with the danger of developing MS, other than the precise reason still remains unidentified. Among the well-recognized environmental risk factors in MS were EBV, smoking, and vitamin D deficiency. The risk of developing MS enlarged by infectious mononucleosis, which is caused by delayed primary infection by EBV. Potentially the EBV acts together with both genetic and additional environmental risk factors to amplify receptiveness to and severity of MS disease [12]. A number of studies communicate EBV with MS [13,14], while others locate no association [15,16]. One of the most consistent pieces of evidence is the finding of elevated antibody titers against EBNA-1 antigen in the blood, both pre- and post-onset of the disease [17].

Several hypotheses explain the mechanism of EBV involvement in MS pathogenesis. One of these is molecular mimicry hypothesis, whereby EBNA-1-specific T cells from MS patients are cross reactive to myelin antigen [18]. The presence of an antigen such as the myelin basic protein (MBP), peptide which is derived from the myelin sheaths surrounding an axon having a homology to EBV viral proteins. Myhr et al., have illustrated molecular mimicry of viral EBNA-1 to MBP that could prompt T cell autoimmunity to myelin sheaths. For this reason, one of the most relevant non-self-antigens that is thought to induce MS is EBNA-1(19).

In the current study, EBNA-1 IgG antibody was positive in 51.7% (62/121) of MS patients and 39.2% (47/121) of controls. To the best of our knowledge, there are two previous studies regarding the seroprevalence of EBNA-1 IgG in Iraq, the more recent study carried out by Abd Al Kareem and Abd in 2020 among the Iraqi female patients with MS who were admitted to Clinic of MS in Neuro-Science Hospital in Baghdad and they reported that mean serum level was significantly higher in female patients than in the healthy controls [11], another study also show significantly higher level in MS patients than the healthy controls (Bakir et al., 2017), which were conducted in Rizgary Teaching Hospital in Erbil, Iraq [10].

The significantly higher seropositivity of EBNA-1 IgG in the MS patients than in the controls is in accordance with other studies [20,21], however, other studies, (Banwell et al., 2007), which was carried out on children, failed to manifest any relationship between the virus and MS [22]. The median level of EBNA-1 IgG for MS patients and controls were 81.08 U/ml and 67.73 U/ml respectively, which was significantly higher in MS patients in the current study, as reported by other studies which have showed significantly higher IgG level in MS patients [10,11,23,24], while others found no significant difference [25,26].

Concerning the association between EBNA-1 IgG results and duration of MS disease, the current study revealed higher serum level and seropositivity of EBNA-1 IgG in MS patients during first two years of disease and this result is in accordance with other study [11], this is indicative of the role of the viral antigen in the early stages of the disease which could be the triggering factor of MS. And due to this triggering factor, the relationship linking EBNA-1 IgG titers and number of clinical relapses showed no statistically significant association as shown in the table (5), this result is close to what's mentioned by Bakir et al. in 2017 [10].

On the other hand, in this study, patients with the first-line and second-line treatment showed no significant differences in EBNA 1 IgG levels between each other, while the median level in patients without treatment were higher, this result is comparable to result of study carried out by Abd Al Kareem and Abd in 2020 [11], these patients who were recently diagnosed with MS had very high median IgG titer also supporting the possible triggering factor by the viral antigen.

In addition, the current study observed that median of EBNA-1 IgG titer was significantly higher in females compared to the males (Table 3), MS is a disease of females, the female to male ratio is about 2:1 [27]. In addition, females have higher percentage of IgG seropositivity than males (59.5% in females versus 39.1 in males), as shown in the table (3). Foroutan-Pajoohian et al. in 2018, also have

reported higher seropositivity of EBNA-1 IgG in MS females than males [28], the above data may possibly be correlated with the more robust immune response enhanced by estrogens compared to the immunosuppressive function of androgens. Females have a better humoral immune response than males, as manifested by higher titers of serum immunoglobulin, and a larger antibody response to a variety of antigens after immunization [29]. However, Bakir et al., in 2017, Kreft et al., in 2017 show no statistically significant difference of mean EBNA-1 IgG titer according to patients' sex [10,23].

The present study clarified statistically significant differences in EBNA-1 IgG titer and seropositivity between MS patients and controls among age groups (<20) and (20-30) years as compared to the controls. This result was somewhat supported by Kreft et al., in 2017 who found that age sampling was significantly correlated with EBNA-1 IgG titer [23].

The current study aimed to investigate the role of EBV in the MS pathogenesis, either as a triggering or initiating factor through the primary active infection, or have a role in initiating relapses through the reactivation from the latent state. Because of higher level and seropositivity in the younger age groups and in the patient who haven't start treatment yet (newly diagnosed) and because there was no significant association in EBNA-1 results with number of relapses. This result may indicate that the EBV could have an important triggering role in MS disease.

Results of EBNA-1 IgG antibodies indicate that the virus could have an indirect effect in development of MS mainly through cross-reacting EBNA-1 IgG antibodies which were significantly higher in MS patients as compared with control subjects. Therefore, it is possible to move towards the use of immune therapy to reduce autoimmunity due to the virus.

In conclusion, EBNA-1 could have an important triggering role of MS because of significantly higher levels both quantitatively and qualitatively in MS patients than in controls, especially at younger age groups, early stages of the disease (in those who haven't start treatment yet), and in the female sex.

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Author contribution

Ali: did the laboratory work and write the article. Dr. Al-Obaidi: Supervision of the study and final editing of the manuscript. Dr. Al-Mashta: consultant and examined the patients.

Conflict of interest

Authors declare that there is no conflict of interest.

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Molecular and Phylogenetic Detection of Torque Teno Virus (TTV) Among Hemodialysis Patients: A Single Center Study

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Abstract

Background Patients on long-term hemodialysis are at a higher risk of contracting blood transmitted infections because of their weakened immune systems and frequent interaction with blood, blood products, equipment, and contaminated surfaces. This making them an important population to study the clinical and epidemiological consequences of newly discovered infections like torque teno virus (TTV).

Objective To investigate the frequency of TTV in patients undergoing hemodialysis by molecular method and to find out any association with risk factors and liver function tests.

Methods This cross-sectional study conducted on (100) patients whom attending Hemodialysis Unit at Al-Imamein Al-Kadhimein Medical City in Baghdad, Iraq. The sample of the study consist of (58) males and (42) females, their mean age is (49.97±4.97 SD) years, (50) with viral hepatitis, while (50) without viral hepatitis, for a period from November 2020 to March 2021. Nested polymerase chain reaction (PCR) was used to detect TTV-DNA. TTV genogroup was determined by Sanger sequencing and phylogenetic tree construction.

Results TTV DNA was detected in 81% (81 out of 100) of hemodialysis patients, respectively. However, no significant association was found between demographic data, clinical characteristics and risk factors with TTV infection.

Conclusion This study showed high prevalence of TTV in hemodialysis patients but didn't play a role in liver injury among these patients. Also, based on phylogenetic analysis of the untranslated region (UTR), genogroup-3 was found to be the most prevalent in hemodialysis patients.

Keywords Torque teno virus, hemodialysis, risk factors, liver function, sequencing, phylogenetic tree

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List of abbreviations: ALP = Alkaline phosphatase, ALT = Alanine transaminase, AST = Aspartate aminotransferase, HD = Hemodialysis patients, PCR = Polymerase chain reaction, TSB = Total serum bilirubin, TTV = Torque teno virus, UTR = Untranslated region

Introduction

In addition to well-known (A to E) hepatitis viruses, there is growing evidence that newer hepatitis viruses may exist and have a role in this disease [1-3]. Torque teno virus (TTV) was first discovered in Japan 1997 from serum of patients with post transfusion non-A to G hepatitis who developed raised alanine aminotransferase level (ALT) [4]. This virus was designated as (T.T) initially from the first patient from whom the virus was isolated [5]. It has a circular DNA genome with a negative strand, but a significantly large genome size [6]. At first, TTV got classified as part of the family circoviridae, genus anellovirus [7]. It was later classified as a member of the Anelloviridae

family [8]. The TTV genome sequence is very diverse in nature and many genetic variants have been detected by using high conserved untranslated region (UTR) as a specific primer for the detection of DNA. Interestingly, the coding regions of TTV are less conserved than the UTR. For instance, the coding region of open reading frame 1 (ORF 1) contains three hypervariable regions (HVRs) in tandem. Variability within ORF 1, which is believed to code for the TTV capsid protein, may be crucial to evasion of the host immune system [9]. According to phylogenetic analysis, TTVs belong to a broad ancestral tree of five important genogroups, and the first and second of them are most prevalent worldwide [10].

TTV is thought to be transmitted by blood transfusion, and its frequency is mainly associated with populations with a history of blood product transfusion [11,12]. Moreover, there are two groups highly associated with TTV virus, these groups are patients undergoing hemodialysis (HD) and intravenous drug users [13]. TTV has been detected in the sera of persons who have not had a blood transfusion, indicating that additional routes of transmission may exist [14]. Virus detection by genogroup-specific polymerase chain reaction (PCR) in serum samples from various geographic regions (China, Canada, Korea, Spain, France, Thailand and the USA) has shown a prevalence ranging from 33% to 100% [15]. The TTV DNA frequency in the Italian HD unit (41.7%) was greater than in the healthy population (10.7%) [16]. While in Indian dialysis patients the universality of TTV DNA was (83%) [17]. In 1999, Gallian et al. showed that the general seroprevalence of TTV was 28 % in HD patients in France [18], on the other hand Kheradpezhou et al. suggested that the seroprevalence was 9.3% in HD patients [19]. There are many reasons behind the higher prevalence in developing countries, which include socioeconomic factors, bad infection control measures and the higher prevalence of the virus among general population (20).

This study aimed to determine the frequency of TTV DNA in hemodialysis patients, as well as, estimation of demographic and clinical data. Also, to determine TTV genogroup prevalence in those patients.

Methods

Subjects

Study conducted on one hundred patients whom attending Hemodialysis Unit of Al-Imamein Al-Kadhimein Medical City to undergo hemodialysis from November 2020 to March 2021. A questionnaire was used to collect data from patients which include sex and age of patients, hepatitis B virus (HBV) and hepatitis C virus (HCV) status, underlying medical condition, history of kidney transplant, previous surgery, marital status, duration of hemodialysis, blood transfusion and number of blood transfusions. In addition, information of liver function test was obtained at the time of study from patient's laboratory report. The ethical approval of the research project was provided by the Institutional Review Board (IRB) at Al-Nahrain University on 20th September 2020 (No. 20200977).

Specimens' collection

Five (5) ml whole blood was drawn from each patient prior to starting hemodialysis in sterile gel tubes and allowed to clot at 25°C for one hour, then centrifuged at 3000 rpm for 10 minutes, serum samples were divided into aliquots in sterile eppindrof tubes which then stored at -20°C until be used.

Isolation of viral DNA

To detect the presence of TTV nucleic acids, DNA was extracted from 200 µl aliquots of serum using the viral nucleic acid extraction kit II (Geneaid, Taiwan) for viral nucleic acid purification from cell-free samples following the manufacturer instructions.

PCR detection of TTV DNA

For detection of TTV DNA, nested PCR specific for detection of sequences included in the UTR region (primers NG034/NG147, and NG133/NG132) as reported by Sarairah et al.

[21] and in the ORF-1 region (N22) (primers NG059, NG063 and NG061) according to Rinonce et al. [22]. The reason behind chosen two regions to amplify is that N22 region is better to the study the genetic variability, however, UTR is the most conserved region identified among strains [23]. The PCR reaction (25 µl) for the first and second rounds of PCR for the both reactions have been prepared by adding 5 µl of the template, 1.5 µM of each primer, 12.5 µl of one Taq PCR master mix (Biolabs, USA) and complete the final volume by adding 4.5 µl of nuclease-free water. Positive and negative controls were run with each round of PCR in order for the results to be considered valid under the conditions given. The thermal cycler settings were customized as those described by both Sarairah et al. and Cancela et al. [21,23] with modifications to optimize the result, as shown in the tables (1 & 2). The amplicon obtained in the first and second run of UTR region is equal to 143 and 110 nucleotides, respectively. Whereas for ORF1, it is equal to 286 and 271 nucleotides, respectively [22]. Both second amplicon region for UTR (110 bp) and ORF (271 bp) were observed in 3% agarose gel [21,24], as shown in the figure (1 & 2).

Table 1. Polymerase chain reaction program for both first and second round reaction using UTR region

Step	Temperature	Time	Cycles description	Cycles
1	94˚C	3 min	Initial denaturation	1
	94˚C	30 sec.	Denaturation	
2	60˚C	40 sec.	Annealing	35
	70˚C	30 sec.	Extension	
3	70˚C	5 min	Final extension	1

Table 2. Polymerase chain reaction program for both first and second round reaction using ORF1 region

Step	Temperature	Time	Cycles description	Cycles
1	94 ˚C	3 min.	Initial denaturation	1
	94 ˚C	30 sec.	denaturation	
2	55 ˚C	45 sec.	Annealing	40
	72 ˚C	45 sec.	Extension	
3	72 ˚C	7 min.	Final extension	1

Quality control

Positive and negative controls were run with each round of PCR in order for the results to be considered valid under the conditions given. Positive control was TTV-Ag positive samples by enzyme-linked immunosorbent assay (ELISA). While negative control was consisted of the reagents that used to prepare the PCR amplification mixture without TTV-DNA and treated like a sample.

Sanger sequencing and phylogenetic tree construction

The PCR amplicons of TTV-UTR region (110 bp) of 10 samples were commercially sequenced from their reverse termini following the guidelines manuals of the sequencing company (Macrogen Inc. Geumchen, Seoul, South Korea). By comparing the obtained DNA sequences of the local samples with the

recovered DNA sequences of the national center for biotechnology information (NCBI) database, the virtual positions and other information of the recovered PCR were recognized. The detected variants were compared with their neighbor homologous reference sequences using the NCBI-BLASTn server. After that, a traditional rectangular tree comprising the detected variant was created by the neighbor-joining method and annotated as a cladogram tree using the iTOL suit [25]. To know the exact identity of the genogroup of local TTV isolates, many references sequences of each TTV genogroup was represented, from genogroup-1 to genogroup-5.

Statistical analysis

Analysis of data was performed by using statistical package for social sciences (SPSS). Comparison is obtained by the use of Chi-square (χ2-test), whereas, numerical data presented as mean, standard deviation and evaluated by using independent sample T-test. Analysis of variance (ANOVA) was used to compare the means of more than two independent groups. The P-value same as or below 0.05 has been considered statistically significant, below 0.01 has been recommended highly significant, and above 0.05 was considered non-significant.

Results

Frequency of TTV based on 5-UTR A and ORF-1

One hundred (100) serum samples have been tested for the TTV DNA by nested PCR as described by Sarairah et al. and Cancela et al. [21,23]. The present study indicated that 81% of hemodialysis patients tested positive for TTV DNA for UTR region as illustrated in the figure (1). While only 1 out of 100 (1%) samples was positive for ORF-1 region as shown in the figure (2).

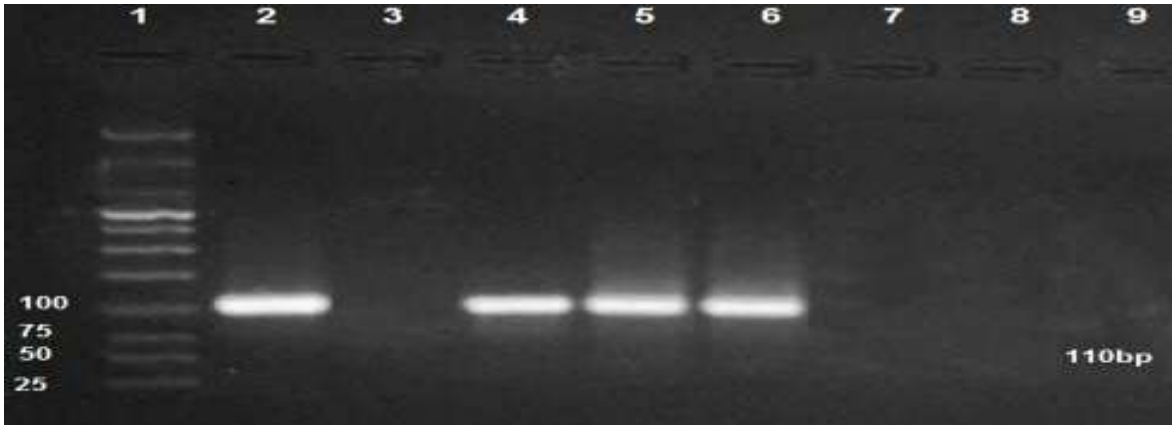

Figure 1. Gel-electrophoresis of second-round PCR products (5-UTR region) using 3% agarose in Tris-acetate-EDTA (TAE) buffer. Lane1, (25bp DNA marker), Lane2: positive control for amplification, Lane3: negative control for amplification, Lane 4,5,6: positive samples, and lanes 7,8,9: negative samples

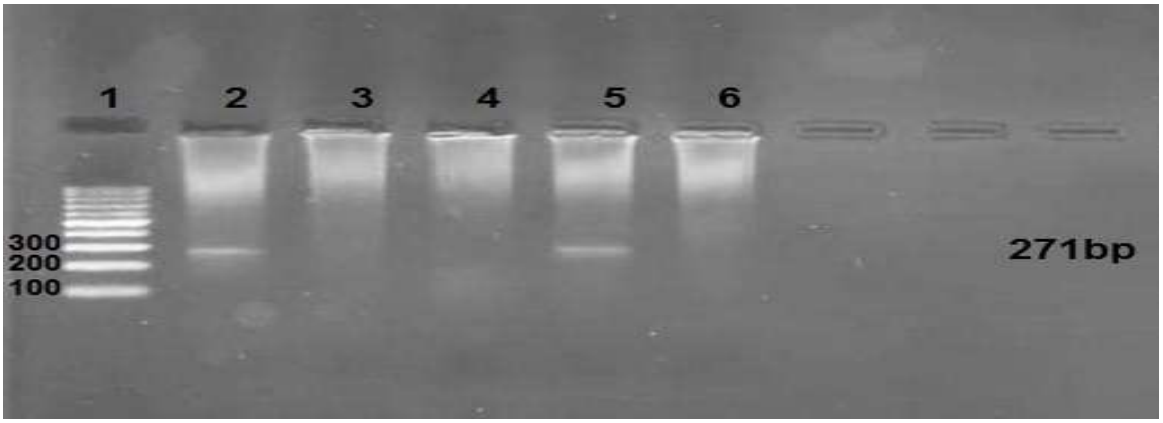

Figure 2. Gel-electrophoresis of second-round PCR products (ORF-1 region) using 3% agarose in TAE buffer. Lane1, (100bp DNA marker), Lane2: positive control for amplification, Lane3: negative control for amplification, Lane 4 and 6: negative samples, and Lane 5: positive sample

Demographic data, clinical characteristic and risk factors

This study showed no significant association between age, sex, diabetes mellitus (DM), hypertension (HT), history of kidney transplantation, history of previous surgery, marital status, hemodialysis duration, history of blood transfusion, number of blood transfusions, viral hepatitis and TTV infection.

Regarding co-infection with hepatitis, current study showed that 50 (50%) out of 100 hemodialysis patients were tested negative to hepatitis B virus (HBV) and/or hepatitis C virus (HCV) and 39 (78%) of them had TTV infection. While the remaining 50 (50%) out of 100 of hemodialysis patients had HCV &/or HBV. TTV was detected in 1 (50%) out of 2 of hemodialysis patients who had co-infected with HBV and 38 (84.4%) out of 45 of hemodialysis patients who had co-infected with HCV. In addition, three patients had triple infection with HBV, HCV and TTV. However, current study showed that there was no significant association between these viruses and TTV status, as shown in the table (3).

Table 3. Demographic data, clinical characteristic, risk factors and TTV status

Parameters		Total No. (%)	TTV- DNA Status (UTR region) Negative No. (%)	Positive No. (%)	*P*-value
Age (mean± SD) year		100 (100%)	48.53±14.10	50.31±15.23	0.634
Age groups (years)	<31	12 (12%)	2 (16.7%)	10 (83.3%)	0.439
	31-40	17 (17%)	3 (17.6%)	14 (82.4%)	
	41-50	17 (17%)	6 (35.3%)	11 (64.7%)	
	51-60	30 (30%)	5 (16.7%)	25 (83.3%)	
	> 60	24 (24%)	3 (12.5%)	21 (87.5%)	
Sex	Male	58 (58%)	14 (24.1%)	44 (75.9%)	0.124
	Female	42 (42%)	5 (11.9%)	37 (88.1%)	
DM	No	67 (67%)	14 (20.9%)	53 (79.1%)	0.491
	Yes	33 (33%)	5 (15.2%)	28 (84.8%)	
HT	No	12 (12%)	0 (0.0%)	12 (100%)	0.074
	Yes	88 (88%)	19 (21.6%)	69 (78.4%)	
History of kidney transplant	No	100 (100%)	19 (19.0%)	81 (81.0%)	–
	Yes	0 (0%)	0 (0.0%)	0 (0.0%)	
History of previous surgery	No	52 (52%)	10 (19.2%)	42 (80.8%)	0.951
	Yes	48 (48%)	9 (18.8%)	39 (81.2%)	
Marital status	No	13 (13%)	2 (15.4%)	11 (84.6%)	0.722
	Yes	87 (87%)	17 (19.5%)	70 (80.5%)	
Hemodialysis duration (year)	< 1	3 (3%)	2 (66.7%)	1 (33.3%)	0.103
	1-3	28 (28%)	7 (25%)	21 (75%)	
	3-5	23 (23%)	4 (17.4%)	19 (82.6%)	
	≥5	46 (46%)	6 (13%)	40 (87%)	
History of blood transfusion	No	34 (34%)	4 (11.8%)	30 (88.2%)	0.186
	Yes	66 (66%)	15 (22.7%)	51 (77.3%)	
No. of blood transfusions (time)	<1	44 (44%)	6 (13.6%)	38 (86.4%)	0.476
	1-4	38 (38%)	9 (23.7%)	29 (76.3%)	
	≥4	18 (18%)	4 (22.2%)	14 (77.8%)	
Virology	Negative	50 (50%)	11 (22%)	39 (78%)	0.444
	HBsAg	2 (2%)	1 (50%)	1 (50%)	0.259
	HCV-Ab	45 (45%)	7 (15.6%)	38 (84.4%)	0.427
	HBV & HCV	3 (3%)	0 (0.0%)	3 (100%)	0.394

DM: diabetes mellitus; HT: hypertension. HBsAg: hepatitis B surface antigen; HCV: hepatitis C virus

Liver function tests

This study shows that there is no significant difference between TTV positive and TTV negative hemodialysis patients in the mean serum level of liver function enzymes such as alanine transaminase (ALT), aspartate Aminotransferase (AST), total serum bilirubin (TSB) and alkaline phosphatase (ALP), as shown in table (4).

Table 4. Serum level of liver enzymes and TTV DNA Status

*Biochemical test (Mean± SD)	TTV-DNA Status		*P*-value
	Negative	Positive	
ALT mg/dl	22.37±14.01	17.88±15.72	0.256
AST mg/dl	16.68±7.73	20.03±15.70	0.370
TSB mg/dl	0.41±0.22	0.35±0.18	0.252
ALP mg/dl	156.74±119.25	201.75±195.35	0.339

Normal value of liver enzymes: ALT= (10-49 mg/dl), AST= (<34 mg/dl), TSB= (0.3-1.2 mg/dl), ALP= (40-130 mg/dl)

DNA Sequencing of 5-UTR A amplicons

Alignments were done by NCBI-Blastn pairwise of the ten local TTV isolate with the most corresponding viral sequences from NCBI that indicated high similarity with variable TTV isolates from America, Europe, and Asia. Local TTV isolates were assigned as (S1 to S10), both S1 and S2 samples had high similarity with the isolate TTVMY02 from Malaysia (GenBank acc. no. MN116509.1). S3, S6, S7, and S8 showed a high similarity with the isolate TUS01 from USA (GenBank acc. no. AB017613.1). S4 and S9 samples showed high similarity with the isolates P10-1 and CK001, respectively which from American and Indian (GenBank acc. no. KT163893.1and KM596845.1). Both S5 and S10 samples were highly similar to the isolated TTV-HD24a and T520, respectively which from two German sources (GenBank acc. no. FR751506.1 and GU722347.1). The current result showed that six nucleic acid substitutions mutation and only one insertion mutation that distributed in the investigated fragments of the local isolate compared with reference sequences according to the figures (3 & 4). The S4 sample observed C48A, T45C and Gins43-44, the S5 sample observed T47G and A41G, while S9, S10 observed A30G and A29C, respectively.

Phylogenetic tree

In the present study, phylogenetic tree was generated using neighbor-joining method, which was based on the 50-UTR A region. This tree constructed by utilizing 22 NCBI relative reference sequences belonging to the significant five TTV genogroups (genogroup-1 to genogroup-5). Within this tree, 4 representatives TTV reference sequences for each of the genogroup 1, 2, 4, and 5 were incorporated. Meanwhile, 6 representatives for the genogroups 3 were incorporated due to its higher variability and availability among TTV. In addition, 10 local TTV isolates were incorporated within this tree.

It was found that the local isolates S1, S2, and S9 were incorporated within the clade of genogroup-2. It deserves to note that both S1 and S2 were equally suited in the vicinity of the KAV isolate (GenBank acc. no. AF435014). Meanwhile, the S9 sample was positioned in the vicinity of two TTV isolates, Kt-08 and Kt-010F. In addition, it was found that all the rest samples were incorporated within the clade of the genogroups-3.

Within the genogroups-3, these viral isolates were distributed into two main positions. It was found that the S3, S6, S7, and S8 were positioned in the vicinity of the TUS0 isolate. Likewise, the S3, S6, S7, and S8 were also respectively positioned in the vicinity to the isolate SENV-C of the same clade. Furthermore, it was found that S4, S10, and S5 were respectively suited in the vicinity to the isolate SANBAN, as shown in table (5) and figure (5).

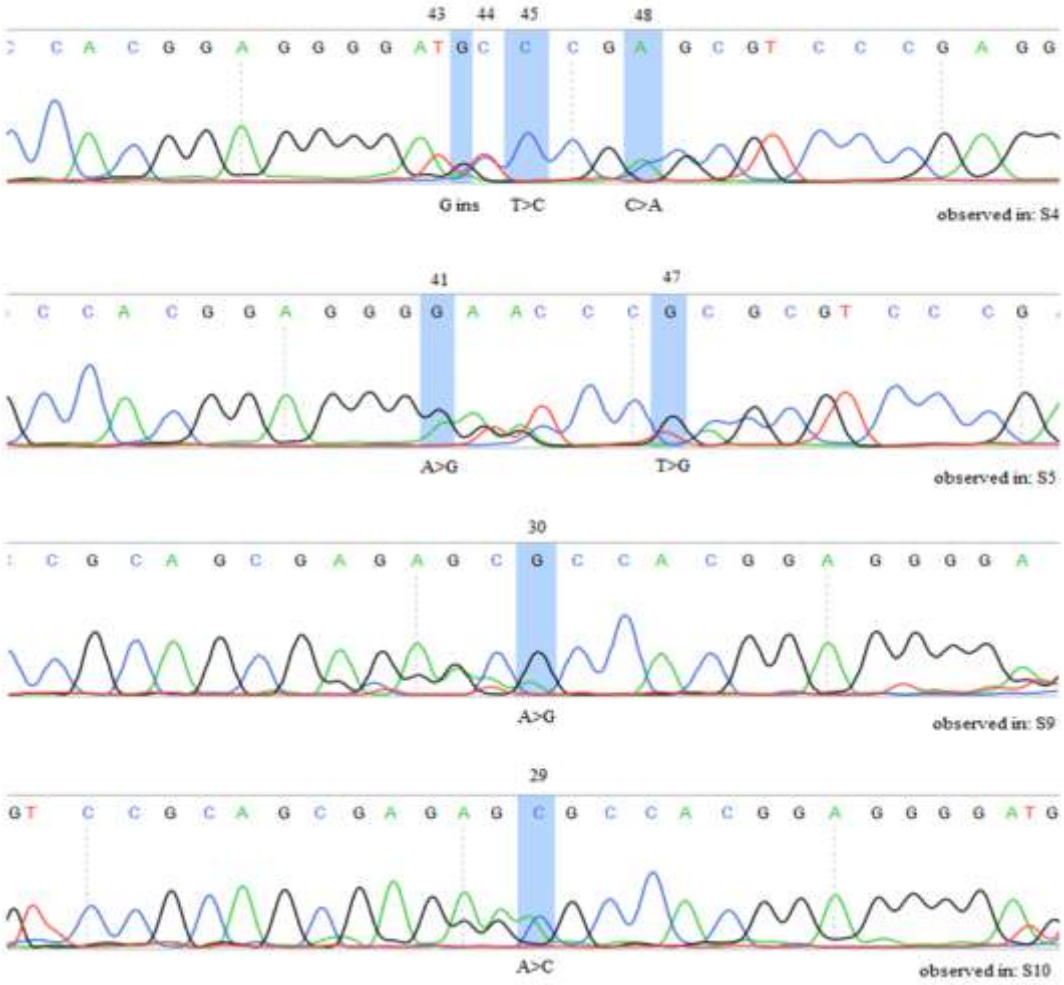

Figure 3. The chromatogram profile of the observed genetic variants of the UTR locus within the TTV viral isolates. The identified substitution mutations are highlighted according to their positions in the PCR amplicons. The letter "ins" refers to "insertion mutation", while the symbol ">" refers to "substitution" mutation. The letter "S" refers to the sample code

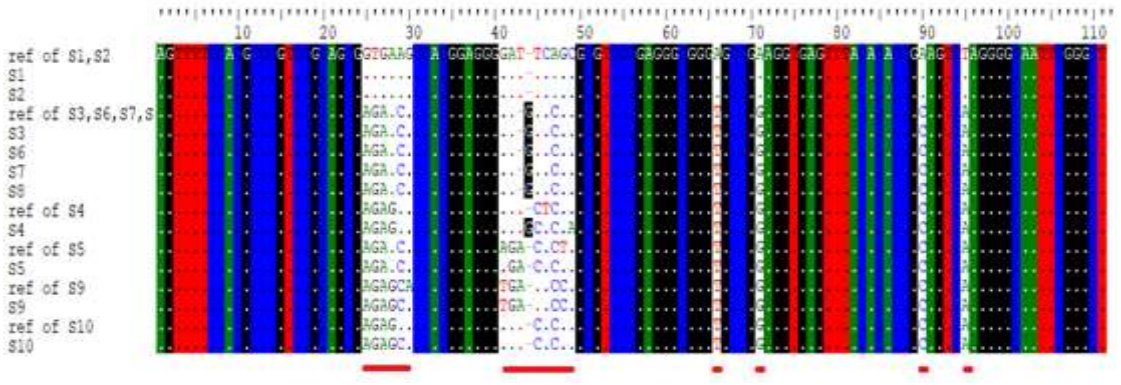

Figure 4. The determination of TTV DNA sequence variability regions in TTV DNA sequences via direct DNA alignment. the red arrows indicated the variable regions, while the colored boxes indicated the conserved regions. The symbol "ref" refers to the NCBI reference sequences, while "S" refers to sample code

Table 5. TTV reference isolates representative of the 5 major genomic groups

Isolate	GenBank acc. no.	Genogroup	Reference
JA20	AF122914.3	1	Erker et al., 1999 [26]
T3PB	AF247138.1	1	AbuOdeh et al., 2015 [27]
JA10	AF122919.1	1	Erker et al., 1999 [26]
JA4	AF122917.1	1	Erker et al., 1999 [26]
Kt-08	AB054647.1	2	AbuOdeh et al., 2015 [27]
Kt-010F	AB054648.1	2	AbuOdeh et al., 2015 [27]
ViPi04	DQ361268.1	2	Maggi et al., 2006 [28]
KAV	AF435014.1	2	Heller et al., 2001 [29]
SENV-D	AX025730.1	3	Okamoto et al., 2001 [30]
TJN01	AB028668.1	3	AbuOdeh et al., 2015 [27]
SENV-E	AX025761.1	3	Okamoto et al., 2001 [30]
SANBAN	AB025946.1	3	AbuOdeh et al., 2015[27]
SENV-C	AX025718.1	3	Okamoto et al., 2001[30]
TUS01	AB017613.1	3	Okamoto et al., 1999 [5]
TTVyon-LC011	AB038622.1	4	Takahashi et al., 2000 [31]
TTVyon-KC009	AB038621.1	4	Takahashi et al., 2000 [31]
TTVyon-KC197	AB038624.1	4	Okamoto et al., 2001 [30]
TTVyon-KC186	AB038623.1	4	Okamoto et al., 2001 [30]
BIS8-17	GU797360.1	5	Hussain et al., 2012 [32]
JT34F	NC_014076.1	5	AbuOdeh et al., 2015 [27]
CT44F	NC_014075.1	5	AbuOdeh et al., 2015 [27]
CT39F	AB064604.1	5	Peng et al., 2002 [33]

Discussion

TTV infection was found to be very common in people who suffered from idiopathic fulminant hepatitis and those suffering from cryptogenic chronic liver diseases including liver cirrhosis, chronic hepatitis and hepatocellular carcinoma [34]. On the other hand, researchers found that the frequency of TTV infection was the same in patients suffering from liver disease including assorted cases of non-B and non-C liver diseases, and also in few healthy individuals. Moreover, they settled that TTV does not cause harm to the liver [35]. In the present study, the prevalence of TTV-DNA was 81 out of 100 patients (81%) based on TTV 5-UTR A region amplification. While, only one was detectable by ORF-1 (N22 region) amplification. This results in agreement with Kenarkoohi et al. who found that TTV prevalence was 92% by using UTR primers and was 5% when using ORF primer [36]. One of the reasons for such results is that UTR primers is more conserved than N22 primers. Another reason could be that UTR PCR can detect more TTV genotypes than N22 PCR which has limited detection [37,38]. In addition, the failure to amplify the N22 region could be attributed to the extremely high variability observed in these strains, which indicated that they may contain new variants [23].

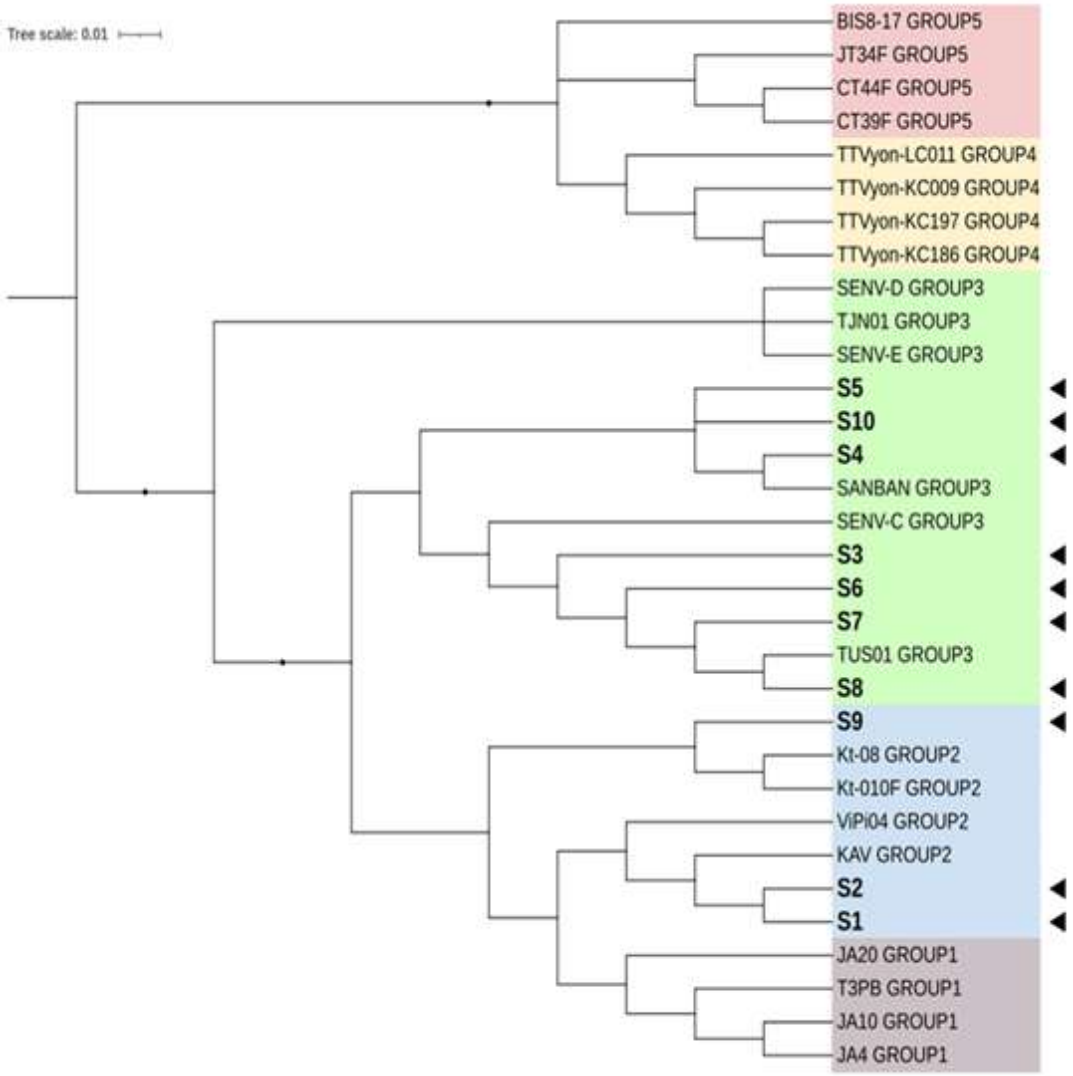

Figure 6. The comprehensive phylogenetic tree of genetic variants of the 5'-UTR locus within ten Torque teno viral isolates. The variably colored numbered refer to the specific genogroup incorporated. The scale of the left portion of the tree refers to the degree of phylogenetic positions among the tree categorized viral organisms. The symbol "S" refers to the code of the investigated samples

Among the current hemodialysis patients, 58 (58%) were males and 42 (42%) were females. Their mean age was (49.97±4.97 SD) years ranging between 14 and 80 years. Considering age of studied population, most of the TTV-DNA positive individuals were with mean age of (50.31±15.23) years according to the table (3). In the current study, there was no meaningful difference between the mean of age between TTV positive and negative HD patients which is close to what's mentioned by Irshad et al. [39]. On the other hand, disagree with Hassuna et al. who showed that there is a correlation between TTV viremia and age [40]. Regarding sex of studied patients, the higher rate (88.1%) of TTV DNA infection was in female rather than male (75.9%) with no significant association between sex and TTV DNA-status, as shown in table (3). These results are in accordance with Mohamed et al. (2017) who showed that the percentage of patients who were positive for TTV as (66.7%), out of

these patients, males were (3.7%) and females (6.1%) with no significant difference in TTV infection between males and females [41]. And these findings are disagreed with Takemoto et al. (2015) who showed that the majority of dialysis patients were males (55%) with a mean age of 53.8 years [42]. In addition, there is no significant statistical association between TTV and clinical characteristics or risk factors according to the table (3). These results are in agreement with Akbari et al. (2018) who observed that there is no significant relation between TTV infection and hypertension [43]. Another study also in accordance with the current findings done by Gallian et al. who found that the TTV prevalence in HD diabetic patients was not significantly higher than that detected in a diabetic patient without renal disease [18]. While, a study done by Spandole-Dinu et al. founded that there is a significant higher level of anelloviral DNA in type 2 diabetes mellitus (T2DM patients) than controls [44].

It is worthy to note that there is no one of the patients in this study underwent a kidney transplant so the relation between TTV infection and kidney transplantation is not known, because of the small sample size due to the fact that during the coronavirus pandemic, researchers were only able to analyze one hemodialysis center.

Regarding the surgical history, the present study showed that there was no noticeable significant association between the positivity of TTV infections and people who undergone surgery in agreement with Khudair et al. [1]. In addition, results showed no association between marital status and TTV infection in consistent with Yazici et al. who found that the TTV was not statistically significant between sexually transmitted risk groups and control group [38]. Patients in hemodialysis treatment are with major concern of infection [45,46]. This susceptibility is increased by their compromised clinical state like immune status and psychological condition especially long duration on HD as well as their extended and frequent exposure to a variety of potential risks, such as blood transfusion [45,47]. Considering the hemodialysis duration, the current study showed a non-significant difference between the TTV infections and the period of hemodialysis. This is near to other study done by Irshad et al. who indicated that TTV infection was not significantly associated with duration of hemodialysis [39]. Although nosocomial infection still may play an important role, but present study can't be ruled out that TTV had other transmission routes such as faeco-oral route, salivary droplet, sexual transition, breastfeeding [48,49]. While Jahromi et. al. reported a significant relation between TTV infection and the period of hemodialysis [50]. Importantly, there was no significant relation between the TTV infection and blood transfusion in hemodialysis patients in accordance with Irshad et al. study [39]. The use of erythropoietin to treat renal anemia resulted in a significant reduction in blood transfusions; however, infections in hemodialysis units can still occur in the absence of other parenteral risk factors [51,52].

The co-infection of TTV and HBV or HCV is common [2,53,54]. Although there was no significant association between hepatitis and TTV status in present study. However, the results showed, that 84.4% of HD patients who infected with HCV were TTV positive and 50% of them who had HBV were infected with TTV, table (3). This result in agreement with Najafimemar et al. who found that TTV-DNA was detected in 54% with chronic hepatitis B infection [55]. Also, this study is close to what's mentioned by Magu et al. who detected TTV DNA in 77% of HCV patients [42]. Furthermore, in Iraq, TTV-Ag was detected in 89 of the HBV-positive patients and in 30.8% of the HCV-positive patients [2]. The current data disagree with Chattopadhyay et al. who showed that among TTV-positive patients, HCV co-infection was absent [17]. Also, another study found the TTV-DNA in 7% of HBV positive patients [23]. In addition, three patients in current study had triple infection with HBV, HCV and TTV. These results are logic since these viruses share the parenteral route of transmission.

According to table (4), there were no significant difference between the level of liver function tests in TTV positive and negative HD patients. This suggests that TTV presence did

not cause severe damages to the liver. This is similar to study done by Irshad et al. who stated that the HD patients with TTV infection did not necessarily have liver dysfunction [56]. Another study by Fabrizi et al. suggested that there was a significant decrease in the concentration of ALT and AST in the hemodialysis patients compared to pre-dialysis patients with chronic renal failure [57]. This may be due to hemodialysis patients decreased secretion of Th-1 associated cytokine (INF-γ), but increased secretion of Th-2 associated cytokine (IL-10), resulting in immunological deficiency [49]. These results are clearly agreed with Lemon et al. who recommended that there are harmless viruses referred to as orphan viruses and are beneficial to the body in a way that they maintain homeostasis [58]. These viruses were isolated but not yet associated with any infection, so they are considered "simple guests". Although it may be difficult to attribute the term guest or endosymbiont to viral agents, but they have a characteristic responsible for altering the normal functioning of cells [59].

In the present study, the alignment of local TTV isolates sequences showed the presence of 99% to 100% of homology with the reference sequences from America, Europe and Asia. However, the result of local viral isolates (S1 to S10) showed the detection of six nucleic acid substitutions and only one insertion mutation according to the figures (3 & 4). Phylogenetic analysis showed that of the 10 sequences analysed, 7 genogrouped with genogroup 3 (S3-8, S10) and only 3 with genogroup 2 (S1-2, S9) as in the figure (5). Based on phylogenetic analysis, both S1 and S2 were completely identical (100%), this is may be due to acquiring the infection from the same source of infection. i.e., nosocomial transmission among hemodialysis patients. In addition, the reason for the tilt of the S9 sample toward both Kt-08 and Kt-010F isolates was attributed to the sequence variations observed in this sample.

The spread of viral strains with the same genotypes, could confirm the possibility of virus dissemination in the hemodialysis setting. The homogeneity of these genotypes reflects a nosocomial transmission [60].

In conclusion, the present study concluded a high frequency of TTV among Iraqi hemodialysis patients with prevalent genogroups-3, however, TTV may didn't seem play a role in liver injury as indicated by liver function tests and therefore at this time mandatory screening for TTV may not be recommended for hemodialysis units.

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Author contribution

All authors contributed to this manuscript. Dr. Al-Shuwaikh: design, interpreted and arranged this manuscript, Ali: performed all the laboratory work and implementation of this study as a part of her M.Sc. study, Dr. Manuti helps in clinical aspect and collection of samples.

Conflict of interest

There is no conflict of interest.

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Diode Laser Ablation of Prostate versus Monopolar Transurethral Electro-Resection of Prostate for Treating Symptomatic BPH: A Prospective Study

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Abstract

Background Benign prostate hyperplasia (BPH) can be treated with endoscopic urological procedures, which includes both laser ablation of prostate and transurethral resection of the prostate (TURP).

Objective To determine the advantages and disadvantages of using diode laser procedure in treating patients with BPH in contrast to TURP treatment.

Methods In a prospective non-randomized study at a single center, 40 patients presenting with symptoms of lower urinary tract symptoms attributable to BPH between the ages of 50 to 90 years were enrolled from November 2014 to June 2015. TURP was used in Group A, and transurethral laser ablation of prostate (TULAP) was used in Group B. Outcomes, including International Prostate Symptom Score (IPSS) and higher maximum flow rate (Qmax) were compared at 3 months.

Results At 3 months, patients treated with TULAP had a significantly Qmax than those treated with TURP ($p<0.001$). There was a significantly lower hospital stay for BPH patients treated with the TULAP technique ($p<0.001$). Patients treated with the TULAP procedure had a significantly shorter catheter time ($p=0.001$). There was a non-significant difference in procedure time between the two methods ($p=0.2$). There was a significant increase in prostate-specific antigen (PSA) among those treated with the TURP technique ($p=0.01$).

Conclusion Lower urinary tract problems induced by BPH can be successfully treated by diode laser ablation of prostate. Our findings suggest that diode laser is reliable and efficient when patients are carefully chosen for surgery.

Keywords BPH, Ablation of Prostate, TULAP, TURP

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List of abbreviations: IPSS = International Prostate Symptom Score, TULAP = Transurethral laser ablation of prostate

Introduction

The prostate is glandular and a fibromuscular structure located immediately under the bladder. The average prostate weighs approximately 20 g and includes the posterior urethra, which measures nearly 2.5 cm in length [1]. More than 30% of men over 65 years old have either irritative or obstructive urinary problems as their chief complaints [2]. Lower urinary tract symptoms (LUTS) affect a large percentage of males [3-5]. Although younger men may have LUTS as well, as men age, the frequency, and severity of LUTS increase, while LUTS may vary greatly to a certain degree [6,7]. As populations age, costs associated with LUTS care are expected to rise rapidly, emphasizing the critical nature of comparing the efficacy and costs of conservative and surgical therapies. [8].

LUTS due to benign prostate hyperplasia (BPH) continues to be a significant issue for men in the United States of America; 75.1% of men over the age of 70 have at least one complaint associated with benign prostate hyperplasia (BPH) [9]. BPH was surgically treated in 8.0% of men aged 60 to 69 years but 22.4% of the men above 70 years. [10]. The best care for LUTS must be determined on an individual basis based on clinical results and the level of discomfort caused by symptoms. Surgery is the preferred therapeutic option in complex situations, such as urinary retention, renal insufficiency caused by urinary retention, or bladder calculi [11-14]. However, trials have demonstrated the success of surgical therapy for LUTS [15,16]. LUTS caused by a urethral obstruction are surgically treated with transurethral resection of the prostate (TURP) for prostates less than 80 ml in volume and open prostatectomy for prostates greater than 80–100 ml in volume. Transurethral resection (TUR) syndrome occurs following the intake of irrigating fluid throughout the surgical procedure [17]. Clot retention has been confirmed to occur in approximately 6% of patients following monopolar and bipolar TURP [18,19].

In spite of the advent of various ways, TURP remains the gold standard for the surgical management of BPH [20]. The TURP procedure is divided into four stages: middle lobe resection, paracollicular resection, resection of lateral lobes and ventral parts, and apical resection [21]. A further significant development was video-assisted resection. Monopolar, high-frequency current with a maximum cutting power of 200 watts is used for electro resection [22]. Complications and morbidity associated with this treatment, including loss of blood, altered fluid balance, improper fluid intake, incontinence, and sexual dysfunction, prompted the advancement and evaluation of novel procedures. Innovations such as laser surgery can aid in mitigating further the risks associated with this technically challenging technique [23,24]. Coagulation of prostatic tissues using diode laser through the urethra is the most common technique applied, with excellent homeostasis, minor morbidity, and decreased patient complaints due to obstruction of the urethra and finally improvement of their quality of life [25,26]. Diode lasers produce energy through a particular diode, since the working wavelength of 980 nm is close to the infrared spectrum, it is readily absorbed by water and hemoglobin. This leads to better coagulation and tissue evaporation properties. Visual laser ablation of the prostate (VLAP) and holmium inoculation of the prostate (HoLEP) are two laser procedures. For even more than 15 years, laser technology was often used to treat LUTS related to BPH [27,28]. Laser therapy is progressively seen as an alternative to TURP for surgical treatment of BPH of almost any volume [29]. Diodes are semiconductors capable of producing and releasing monochromatic light. This light is then refracted into a crystal, producing the ultimate wavelength. Diode lasers come in a variety of wavelengths and fiber configurations (side-firing and end-firing) [29-30]. Depending on the wavelength, energy, and sort of laser emission, techniques such as coagulation (photoselective vaporization of the prostate [PVP]), vaporization (PVP), and diode resection, and enucleation are available [30-31].

The primary drawback of these lasers is their near-infrared wavelength, which causes coagulation necrosis due to its precisely established deep spatial intrusion. Dysuria, sloughing, and long-lasting storage effects are caused by this necrotic tissue [32].

The objectives of this study is to define the pros and cons of using diode laser ablation in the management of patients with BPH in contrast to TURP at 3 months following surgery.

Methods

A prospective, non-randomized, research was conducted between November 2014 to June 2015 at a single center at Sulaimani. Forty patients diagnosed with symptomatic BPH, 20

of whom underwent monopolar TURP, and 20 underwent transurethral laser ablation of prostate (TULAP), the choice of the surgery type was according to patients' decision depending on their personal opinion and perspective. Patients' age in group A ranged from 50 to 79 years (76±7), prostate volume 65 to 81 g (71±26.2), International Prostate Symptom Score (IPSS) 11-35 (23±7), quality of Life (QoL) 2.3-5.6 (4.1±0.1), maximum flow rate (Qmax) 8-14 ml/s (11.8±1.9), while in group B, patients' age range was 60-90 years (81±13), prostate volume 83 to 150 g (118.3±47.3), IPSS 12-35 (21±8), QoL 2.1-5.4 (3.9±1.1), Qmax 7-14 ml/s (11.5±2.06). In each case, pharmacological therapy was attempted but resulted in a marginal or non-responsive reaction. Patients were assessed using physical examination, including the digital rectal examination (DRE), IPSS, prostate-specific antigen (PSA), uroflowmetry, and transrectal ultrasonography (TRUS). Outcomes at 3 months following surgery, including IPSS, Qmax, PSA together with complications were compared between the two groups.

Inclusion criteria

Patients complaining of moderate to severe LUTS, as calculated by the IPSS (score ≥ 8), and a Qmax of less than 15 ml/s during flowmetry, with and without substantial post-void residual volume (PVR) as determined by ultrasound. Urine analysis, and blood testing including serum PSA, complete blood count (CBC), prothrombin time (PT), partial thromboplastin time (PTT), international normalized ratio (INR), blood group, renal function tests, and blood glucose level, were also performed for all patients.

Exclusion criteria

An active urinary tract infection at presentation, the presence of a vesical stone, urethral strictures that could preclude the insertion of a rigid 20 F cystoscope, previous TURP or laser treatments, pelvic operation, prostate-specific antigen concentration of more than 10 ng/l or abnormal DRE, medical history of prostate or bladder cancer, evidence of neurogenic bladder dysfunction as confirmed by urodynamic study.

Methods

Before they participated in the present study, all participants were interviewed and fully informed about the procedures and signed a written informed consent.

Both groups of patients who underwent treatment procedures received spinal anesthesia, and the operation was conducted by three surgeons, one of the surgeons who conducted the TURP surgery was also the surgeon performing TULAP, which had been done by him over many years for large number of patients in the same center. Monopolar TURP was conducted using a Storz 25 F resectoscope, and a STORZ ICC 350 generator (Germany) set to 130/50 W (cutting/coagulation mode). Every resection was performed using regular loops and manufacturer glycine-containing irrigating fluid. On the other hand, Prostate ablation was performed on those who experienced TULAP using a diode laser at 980 nm (CERELAS, BIOLITEC, GERMANY) using a 600 nm side-firing and end-firing fiber endowed inside a 1 mm diameter spot, with a 150 W of maximum output power. Irrigation with saline solution or glycine solution (in case of unavailability of the 3000 mL normal saline solution irrigation bags) was performed using a 22 F cystoscope. Ablation was initiated clockwise at the bladder neck by moving the resectoscope farther out and concurrently revolving the laser fiber at a power setting of 140 to 150 W. As with TURP, all prostatic tissue obstructing the prostate was extracted before a fine surgical cavity was created. Regardless of the presence of clear urine or mild hematuria in all circumstances, a 24 F three-way catheter was mounted. A urethral catheter was inserted following the procedure and removed the following day in all cases of TULAP, while 3 to 5 days were needed in cases of TURP, depending on the degree of hematuria. Three months after the operation, postoperative Qmax, PVR, and IPSS with QoL scores were collected and compared between the two groups. The time of the operation and

catheterization were determined and compared for both groups.

Fisher's exact test and non-paired student t tests were used by IBM statistical package for social sciences (SPSS) statistics for windows, version 23, with a p value <0.05 considered as significant.

Results

There were a whole number of forty male patients, with a mean age for those treated with TURP as (76±7 years). About two-thirds of TURP patients were self-employed, and their mean weight was (73±8.9 Kg). The Mean age of patients treated with TULAP was (81±13 years). About two-thirds of TULAP patients were retired, and their mean weight was (85.3±10.8 Kg) as shown in table (1).

Table 1. Baseline criteria of BPH patients treated with TURP and TULAP

Variable	Group A (TURP)	Group B (TULAP)
Age (year)	76±7	81±13
Weight (kg)	73±8.9	85.3±10.8
Prostate size (g)	71±26.2	118.3±47.3
DM	4	3
IPSS	23±7	21± 8
QoL	4.1±0.1	3.9±1.1
Qmax (ml/s)	11.8±1.9	11.5±2.06
PSA (ng/ml)	4.9±2.5	3.2±1.2

DM: Diabetes mellitus, IPSS: International Prostate Symptom Score, QoL: Quality of Life, Qmax: Maximum flow rate, PSA: Prostate-specific antigen

The weight of BPH patients treated with TULAP was significantly higher than those treated with TURP (p<0.001). The prostate size was significantly larger in patients who underwent the TULAP procedure (p<0.001). The Qmax was significantly improved in BPH patients receiving TULAP (p<0.001). Those patients of BPH operated with the TULAP procedure had a significantly shorter hospital stay (p<0.001). Catheter time was greatly decreased in patients undergoing TULAP (p<0.001). No major variation in technique time was found between both the TURP and TULAP therapies. (p=0.2). Regarding IPSS, QoL scores, and PSA for the two groups; there was no significant difference among those parameters for both groups (p>0.05) as shown in table (2).

There was no difference in postoperative complication between the TURP and TULAP procedures (p=0.2). 35% of BPH patients managed by TURP did not experience any complications; the common postoperative complications of TURP were urine retention (due to clot retention or due to a small piece of prostatic chips that obstructed the openings of the catheter (15%), dysuria (35%), hematuria (15%), UTI (10%), epididymo-orchitis (5%), and blood transfusion (10%). 45% of BPH patients treated with TULAP had no postoperative complications; the common postoperative complications of TULAP were urine retention (15%), dysuria (40%), re-insertion of the catheter (10%), urge incontinence (10%), retrograde ejaculation (5%), UTI (5%), and epididymo-orchitis (5%). While no patient treated with TULAP had blood transfusion as shown in table (3).

Table 2. Post-operative (3 months) outcome: prostate size, Qmax, hospital stay, catheter time, procedure time, and PSA between the two groups according to TURP & TULAP techniques

Variable	TURP Mean±SD	TULAP Mean±SD	P value
Prostate size (gm)	23±3.5	15.7±2.3	<0.001
Qmax (ml/s)	17.3±1.19	18.5±1.81	0.4
Hospital stay (day)	2.2±1.1	0.6±0.5	<0.001
Catheter Time (day)	4.7±1.7	1.8±0.4	<0.001
Procedure time (hour)	0.9±0.3	1.07±0.4	0.2
PSA (ng/ml)	3.2±1.92	3.6±1.01	0.3

Qmax: Maximum flow rate, PSA: Prostate-specific antigen

Table 3. Distribution of postoperative complications according to TURP & TULAP techniques

Complication	TURP		TULAP		P value
	No.	%	No.	%	
No	7	35.0	9	45.0	
Urine retention	3	15.0	3	15.0	
Dysuria	7	35.0	8	40.0	
Hematuria	3	15.0	0	0.0	
Re-insertion of catheter	0	0.0	2	10.0	
Retrograde ejaculation	0	0.0	1	5.0	0.2
Urge incontinence	0	0.0	2	10.0	
UTI	2	10.0	1	5.0	
Epididymo-orchitis	1	5.0	1	5.0	
TUR syndrome	0	0.0	0	0.0	
Blood transfusion	2	10.0	0	0.0	

UTI: Urinary tract infection, TUR syndrome: Transurethral resection syndrome

Discussion

Laser surgical therapy of patients complaining of BPH and LUTS achieves comparable results and clinical outcomes similar to TURP [33-37]. However, the concept that TURP could be substituted in favor of laser surgery as the gold standard is not generally recognized owing to a paucity of large-scale trials [32]. In recent episodes, mortality following TURP has decreased significantly in the last few decades to 0.25% [38]. This may be primarily due to improvements in anesthesia and advancements in the technology of TURP [3].

Compared with monopolar TURP, thulium, holmium and diode lasers were associated with better efficacy and fewer complications [39].

Up to 30-40% of patients experience early urge incontinence; nevertheless, late iatrogenic stress incontinence is uncommon (<0.5%). Notwithstanding an aging population (55% of patients are over the age of 70), TURP has low related morbidity (1%) and a mortality rate of (0-0.25%). Bladder neck contractures (0.3-9.2%) and urethral strictures (2.2-9.8%) are the most common late complications. In this study, there was no blood transfusion needed, no TUR syndrome, we have 15% urine retention which is due to clot retention, 10% LUTS, which may be related to catheter blockage, theater infection control, no cases reported as early urethral stricture or bladder neck contracture, may be due to short-duration study.

Additionally, we discovered that laser surgery for the treatment of BPH had a low risk of intraoperative and postoperative complications. Patients who received diode laser therapy did not need withdrawal of anticoagulants or blood transfusions. Additionally, 15% of patients had urine retention, which was due to irritative symptoms, re-catheterization rate was 10%, while in other trials, patients in the diode laser category had a re-catheterization rate of about 17%, this may be attributed to the limited sample size [40-41].

According to Rieken et al., [34] 9.6% of patients who received diode laser therapy needed reoperation for bladder neck closure, opposed to 3.6% of all those who received TURP, whereas there were no such problems in this research. Likewise, although a urethral stricture formed in 5.5% of those receiving diode laser surgery against 0% among those undertaking TURP [42-43], there has been no urethral stricture in this analysis throughout follow-up with either TURP or TULAP, although this may be due to the short duration of follow-up in this study.

In the present study, for those treated with TURP, there was a significant decrease in post-voiding residual volume postoperatively ($p<0.001$), which can be explained by the fact that more than 50% total prostate volume is excised during TURP which leads to immediate post-operative improvement in those parameters [44].

Fagerström et al. [45] in a case study reported that (71%) of catheters were withdrawn during 24 hours, and a further (12%) of catheters were removed within 48 hours in patients treated with TURP. Patients left the hospital with an indwelling catheter if a second attempt to remove the catheter was unfruitful. While in this study, the meantime of catheter removal time in TURP patients is 4.7 days and in TULAP cases is 1.8 days (43 hr) for all cases. Akman et al., [46] reported to have prostate dissection via monopolar transurethral resection (TURP) followed up for 12 months. The mean procedure duration was (58.7 minutes) for monopolar TURP. The incidence of TUR syndrome was 1.4% for monopolar TURP. In the TURP sample, the duration of hospital stay (2.7 days compared with 2.2 days). There were fewer rates of clot retention (0.8% vs 15%) and mean time to catheter removal (2.4 days compared with 4.7 days), which is near to a similar study [47].

Razzaghi et al., [48] reported similar figures to our study; in TURP and diode groups, the operation time was 54.9 vs 60.6 minutes ($P = 0.14$), Foley catheterization time was 88.9 vs 20.1 hours ($P<.0001$) and postoperative hospital stay was 59.9 vs 25.8 hours ($P < .0001$) respectively. Other similar studies done in Iraq and involving laser treatment for BPH showed similar results and any slight differences may be due to difference in type of laser used and the sample size as well as the duration of the follow up [49-51].

Most problems, which arose during the perioperative phase (up to just the end of the first month also for a period of 3 months following procedure) were recorded. Of course, the cost difference between TURP and TULAP may affect the choice of surgical treatment option as the laser is more expensive procedure, that may make it unaffordable option for some patients.

There are limitations in the present study like small sample, single center, no randomization, short follow up that precluded the assessment of long-term complications such as urethral stricture, bladder neck contracture, and erectile dysfunction.

In conclusions, the choice to treat BPH using TURP or Laser can be influenced by patient's factors such as age, co-morbidities, and concurrent anticoagulation. Laser ablation of the hypertrophied prostate has been shown to be a reasonable and reliable surgical procedure for relieving symptoms associated with symptomatic urinary outlet obstruction with comparable results to TURP. However, long term follow up studies are recommended to follow up the TULAP patients to assess long term complications and acceptance by urologists and patients.

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Author contribution

Both authors have contributed to the scientific work and investigations, writing and editing of all data included in this study.

Conflict of interest

The authors declared that they have no conflict of interest.

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Resistance Mechanisms to First Line Drug in *Mycobacterium tuberculosis: A Review*

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Abstract

The emergence of multidrug-resistant (MDR) *Mycobacterium tuberculosis (Mtb)* strains, identified as resistant to at least isoniazid and rifampin, these two drugs form the backbone of the first line drug which is used for tuberculosis (TB) treatment, unresponsive has hampered TB control. As a result of the nearly universal calculation of with half a million new cases of MDR/first line drug TB per year, it is important to keep the database up to date awareness of the processes that contribute to the emergence of MDR *Mtb*. This resistance is produced for a variety of reasons, including genetic, microbiological factors, non-adherence to treatment by patients and/or failures in therapy administration by some referrer medical centre for TB. This review offers a detailed summary of genetic mechanisms that lead to resistant to first line drug therapy used in management of TB as well as up-to-date information on some new aspects lead to such problem.

Keywords *Mycobacterium tuberculosis*, first line drug, anti-TB treatment, World Health Organization, isoniazid and rifampin

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List of abbreviations: DST = Drug susceptibility testing, XDR-TB = Extensively drug-resistant tuberculosis, HGT = Horizontal gene transfer, MDR = Multidrug-resistant, *Mtb* = *Mycobacterium tuberculosis*, PZA = Pyrazinamide, TB = Tuberculosis

Introduction

Tuberculosis (TB), one of the ancient infections known to affect humans, the main target organ of this disease is the lung, but can be disseminated to involve any tissue through the body. The mortality rate increases annually [(1)]. World Health Organization (WHO) reported that around 1/4 of the world's population can presented with latent TB, in which tuberculin skin test give positive, which can progress to active TB [(2)].

Treatment regimen involved streptomycin, isoniazid, rifampicin, ethambutol, and pyrazinamide (PZA), which referred as first-line anti- TB drug, while the second line of anti-TB composed from fluoroquinolones, amikacin, kanamycin, capreomycin, ethionamide, prothionamide, cycloserine, and para-amino salicylic acid [(3)].

One of the most treatment challenged is the appears of drug-resistant *Mycobacterium tuberculosis* (*Mtb*) strain, which is including either un-respond to first-line therapy mainly isoniazid and rifampicin and it is called multidrug-resistant TB (MDR-TB), or if resistant to isoniazid and rifampicin plus resistance to fluroquinolone such as levofloxacin or

moxifloxacin in addition to that it resistant to at least one of the three injectable second-line drugs (amikacin, capreomycin or kanamycin), it refers to extensively drug-resistant TB (XDR-TB) [3].

The burden of the drug resistances TB strain will be growing in last years in the TB endemic countries, many fundamental risk factors may be led to this troubled situation, such as misused of anti TB drug, therapy regimen failure, tuberculosis relapse, difficulty getting a drug susceptibility testing (DST) and in some country's patients did not get drug supply easily [4].

Unluckily, conventional culture methods using egg-yolk-enriched media till know considered the most utilized methods for TB-DST, long time required to get result for this conventional method displease doctors in the management of TB cases, this forces doctors to give treatment to newly diagnosed patients without resorting to TB-DST, despite the presence of newer methods for studying TB-DST, unfortunately the high cost of such newly methods in low and middle-income countries determine their use [4].

Cell wall structure of *Mtb* is complex and diverse it composed from high lipid content, the fraction lipid of this cell wall including cord factor, mycolic lipid, wax D in addition to that the cell envelope of *Mtb* is extraordinarily hydrophobic and forms a remarkably strong permeability barrier, rendering *Mtb* naturally not respond to a broad variety of anti-TB therapy [5].

The resistance of TB bacteria to various treatment mainly to isoniazid and rifampicin, the most effective anti-TB drugs has become one of the global problems and lead to increase morbidity and mortality of this disease [6].

From an evolving viewpoint, *Mtb* use two main genetic strategies to avoid and escape from attack of anti-TB treatment, these genetic methods including mutations in gene(s), which often related to the mechanism of action of the compound, and second strategies is acquisition of DNA coding for resistance determinants by means of horizontal gene transfer (HGT) [7].

Genetic structure of *Mycobacterium tuberculosis*

The chromosomal characteristic of *Mtb* equal to 4,200,000 nucleotides long and the G to C ration is about 65%, this genome comprised from 4000 gene, and one of the essential gene in this genome is genes that code for lipid metabolism which is Which constitutes roughly about 8% of the total of this genome makeup [8].

DNA homology between all *Mtb* complex show 95-100% connections, while in the case of the 16S rRNA gene, it is found in all types of these bacteria, for this reason some researchers suggest this species grouped as a single species [9].

From other hand, l6S-23S rRNA internal transcript spacer (ITS), which is spacer DNA well used for species determination of mycobacteria that due to elevated of variation between species, both in their base length and in their sequence the data that suggest mycobacterium species contain alleles in the ribosomal operon in their genome enhance the potential that a considerable quantity sequence variation exists in these spacer regions, even among strains of the same species [10].

First-line drugs used in the fight against tuberculosis

The battle against TB started since ancient times and is still going on. Various types of drugs have been introduced into the battlefield, and the first-line drugs remain among the most important and ancient weapons in this confrontation for various reasons, including ease of processing and access to them, and that is their ability to deal with disease under different TB pathogenesis process [11].

Effective TB treatment introduce in 1952 and since, through all this time *Mtb* have acquired unresponsiveness to various type of drugs

effect both treatment and control programs. MDR and XDR-TB have emerged due to inappropriate use of anti-TB medications, incorrect prescriptions, poor quality drugs and ending treatment prematurely [12].

The manner to treatment the TB is completely distinct from that for other bacterial infections, *Mtb* required long generation time and have capability for latency which led to decrease the metabolic processing makes it a problematic in therapeutic target [13].

Furthermore, one of most effective scape mechanisms in *Mtb* is granuloma formations, which composed of solid caseous material make the penetration of anti-TB is difficulty and the environmental pH is adequately low to prevent the activity of anti- tuberculosis therapy [14]. Particularly when treatment by using mono-therapy anti- TB, for instance, isoniazid is vital in the initiation of therapy; its bactericidal activity promptly diminishing the viable organism in sputum because it is active mostly toward the TB growing in pulmonary cavities [15]. PZA is only working at low pH, making it preferably suitable for destroying the tuberculosis inside caseous necrotic foci [16]. Rifampin has a great role in destroying the bacteria that are metabolizing slowly in constant, and lead to reduce the infectious agent in the target tissue [17].

The consequent descriptions of first-line anti-TB therapy gave the medical center the basic methods for TB management and control. The subsequent series of trials performed under the supervision of the U.S. Public Health Service, WHO guideline and others produced data lead to that cure rates of over 95% with limited relapse rates were practicable in as little as 6 months, using the first line with the new drug against TB, many countries have seen the virtual eradication of TB [18].

Drug resistance in *Mtb*

One of the important methods lead to developing drug resistance in *Mtb* is mutation in genes, which leads to the influence or disruption of the work drug-activating enzymes, these mutations can take form of single nucleotide polymorphisms (SNPs), insertions or deletions and to lower level, large deletions. *Mtb* differ from other bacteria in that, un-responses to drug are not gained by HGT by mobile genetic elements [17].

There are two major mechanisms lead to drug resistance in *Mtb*, which is primary drug resistance in which bacteria acquired when it is transmitted to a new host, the second mechanisms are secondary drug resistance in which *Mtb* obtainment drug resistance mutations to one or more drugs [19].

Many researchers according to whole genome sequencing (WGS) reported that *Mtb* drug resistance firstly to isoniazid then resistance to rifampicin or ethambutol followed by resistance to PZA and lastly, resistance to second- and third-line drugs. These studies give valuable perception into the evolution of the *Mtb* [20-22].

Mechanisms of resistance to first-line drugs

Isoniazid

Isoniazid belongs to a group of bioreversible derivatives therapy called prodrug, in which must be experience enzymatic and/or chemical conversion in vivo to release the active origin drug, so in the isoniazid drug an activating process takes place through catalase/peroxidase enzyme encoded by the katG gene, after triggered isoniazid lead to prevent formation of mycolic acid by the NADH-dependent enoyl-acyl carrier protein reductase, which is a key enzyme in fatty acid synthesis, this enzyme is encoded by gene called inhA gene [23,24].

The mechanisms that lead to developed unresponsive to isoniazid is mediated by mutation that occur in the katG, inhA gene, the widespread resistance process has been detected is the katG S315T mutation, this mutation causes ineffective isoniazid–NAD product inhibiting the antimicrobial action of isoniazid which is consequently lead to high-rate isoniazid resistance in MDR isolates [25,26].

Machado et al. stated that mutations that take place in the inhA regulatory region and coding region generated high-ranking isoniazid resistance and it's a key step in resistance to ethionamide [27].

In addition to katG, inhA gene mutation, other mutation has been recorded and involved in resistance to isoniazid such as dfrA gene mutation which encodes a thymidylate synthase, furthermore alkyl hydroperoxide reductase C is a type of the peroxiredoxin family possess peroxinitrite reductase activity as well as peroxidase activity hat diminish organic peroxides to their corresponding organic alcohols which encoded by the ahpC gene in mycobacteria [28].

Mutations in the promoter region of the ahpC gene were proposed as representative markers for isoniazid unresponsive. Studies have also reported a variety of mutations were identified in the kasA, oxyR-ahpC and furA-katG in isoniazid-resistant isolates of *Mtb* [29].

However, systematic review reported that mutations in katG and inhA is the play important mechanisms associated with isoniazid resistance, respectively. These common mutations, in association with frequently occurring mutations in the ahpC-oxyR, account for 84% of worldwide phenotypic isoniazid resistance [30].

WGS display proof that isoniazid resistance predates rifampicin resistance, associated with the katG S315T mutation. This carries out this mutation a perfect marker of the pre-MDR phenotype. Globally, the case rate of isoniazid resistance is over growing and is associated with worse outcomes [31].

Rifampicin

When *Mtb* sensitive to anti-TB therapy, rifampicin concerned one of the most effective drugs in first line anti-TB drug, its act actively in both metabolizing and low-metabolizing TB [32,33].

The mechanism of action is initiating when rifampin inhibits elongation of mRNA by binding to the b subunit of the RNA polymerase, which lead to gradual accumulation of mutations in 81-bp rifampicin resistance-determining region, which is found in the gene rpoB (codons 507 to 533), this process caused transcription failure in bacterial cell. The widespread mutations in the rifampicin resistance determining region (RRDR) are reported in codons 526 and 531 and its estimated 62.5% - 81.1% of *Mycobacterium* rifampin-resistant [34].

Cross-resistance to all class of rifamycin antibiotics such as rifabutin and rifalazil have been reported with rpoB mutations and attributable to mutations within the hotspot region, early regions of the rpoB gene and double mutations in codons 516 and 529 [35].

Some investigations reported absence of alteration in the rpoB gene in *Mycobacterium* rifampicin-resistant isolates suggesting other mechanisms of rifampicin resistance [36]. Minh et al. suggested that at external RRDR, rare mutations were reported in *Mycobacterium* isolates [37].

Mtb that resistant to only rifampicin without other agent in first line anti-TB drug such as isoniazid refer as rifampicin monoresistance, and it's not commonly reported strain [38].

Rifampicin mono-resistant TB led to unsuccessful treatment of TB depend on first line anti-TB drugs and any TB regimen without rifampin lead to bad prognosis outcome [39]. The American Thoracic Society, Centers for Disease Control and Infectious Diseases Society of America advisable a 12–18-month regimen for rifampicin mono-resistant Tb with isoniazid, ethambutol, and a fluoroquinolone, with the addition of PZA for the first 2 months [40].

Pyrazinamide

Pyrazinamide (PZA) is a major member of first line anti TB regimen and sometime used as a key component in treatment of rifampicin mono-resistant TB, it is considered as a pro-drug required conversation into pyrazinoic acid (POA) by the pyrazinamidase/nicotinamidase (PZase) enzyme, encoded by the pncA gene [41].

The activity of PZA is thought to be more at an acidic pH (within macrophages) and its effective against dormant or non-replicating (TB lesion) and little activity toward *Mtb* [42,43].
PZA enters bacilli through passive diffusion and is converted into POA by the cytoplasmic PZase encoded by pncA. POA then gets out of the cell through passive diffusion and a deficient efflux mechanism in *Mtb*, upon activated, POA inhibiting membrane transport through break up the bacterial membrane, in an acidic environment, POA is protonated allowing for reabsorption into the cell, resulting in cellular damage [44].
Resistance to PZA is associated with many mutations such as mutations in the rpsA (ribosomal protein I) gene and mutation in aspartate decarboxylase (PanD) However, the mutation in the pncA gene and its promoter region remains the most important one in these mutations and commonest mechanism-initiated pyrazinamide resistance [45].

Ethambutol

Since its introduction as a treatment for TB in 1966 until now, this treatment is still the backbone agent in first line drug used in the management of TB. The anti-TB activity of this agent relating to the ability to prevent formation of mycobacterial cell wall by interaction with arabinogalactan and lipoarabinomannan (LAM) through membrane-embedded arabinosyltransferases — EmbA, EmbB, and EmbC [46].
Some mutant proteins from the Emb family (ABC) have been isolated in ethambutol-resistant *Mtb* which suggest that resistance to ethambutol is mediated via mutations in the Emb family [47]. Safi et al reported that mutations in ubiA gene which encodes for decaprenyl-phosphate 5-phosphoribosyltransferase synthase cause high-level ethambutol resistance when they occur with embB mutations [48].

Streptomycin

Streptomycin, an injectable aminoglycoside therapy, it's one of the first anti-TB agent that has a distinct repressive effect on tuberculous infections. As a result, to initial broad use as anti-TB led to the early emergence of streptomycin resistance [49].
This drug is active against slow-growing bacilli and it have direct effect on bacterial ribosome particularly 30S subunit, when it interacts with S12 and 16S rRNA in form of irreversibly binding via this interaction, streptomycin blocking translation consequently inhibiting protein synthesis [50].
The leading mechanism of resistance to streptomycin is occur through the mutations in the rpsL and rrs genes, encoding the ribosomal protein S12 and the 16S rRNA [51].
There are some studies mentioned that gidB gene which encoding a 7- methylguanosine methyltransferase specific for methylation of the G527 in loop of the 16S rRNA may be undergo a mutation lead to low-level streptomycin resistance [52,53].

Epistasis in *Mtb* drug resistance

Epistasis or interactions between genes, is a situation in genetics in which the impact of a gene mutation is dependent on the presence or absence of mutations in at least one different gene [54]. Epistasis occurs when several mutations interact with each other to express new advantageous traits for an organism and are often necessary for bacteria to modify their fitness cost (fitness in microbiology is the ability of microbes to thrive in a competitive environment. It is often determined by comparing the growth rate in a given environment of a mutant strain with that of its non-mutant isogenic relative [55].
During epistatic interactions, the effect of multiple mutations is greater or less than the effect of the individual mutation and can lead to either beneficial or deleterious phenotypes. According to this concept epistasis is classified as positive epistasis (antagonistic), negative epistasis (synergistic), and sign epistasis [56]

(Figure 1). A study by Borrell et al. has reported the role of positive epistasis in drug resistance development in *Mtb* and they reported that a particular combination of mutations in rpoB and gyrA that conferred resistance to RIF and ofloxacin (OFX) [57].

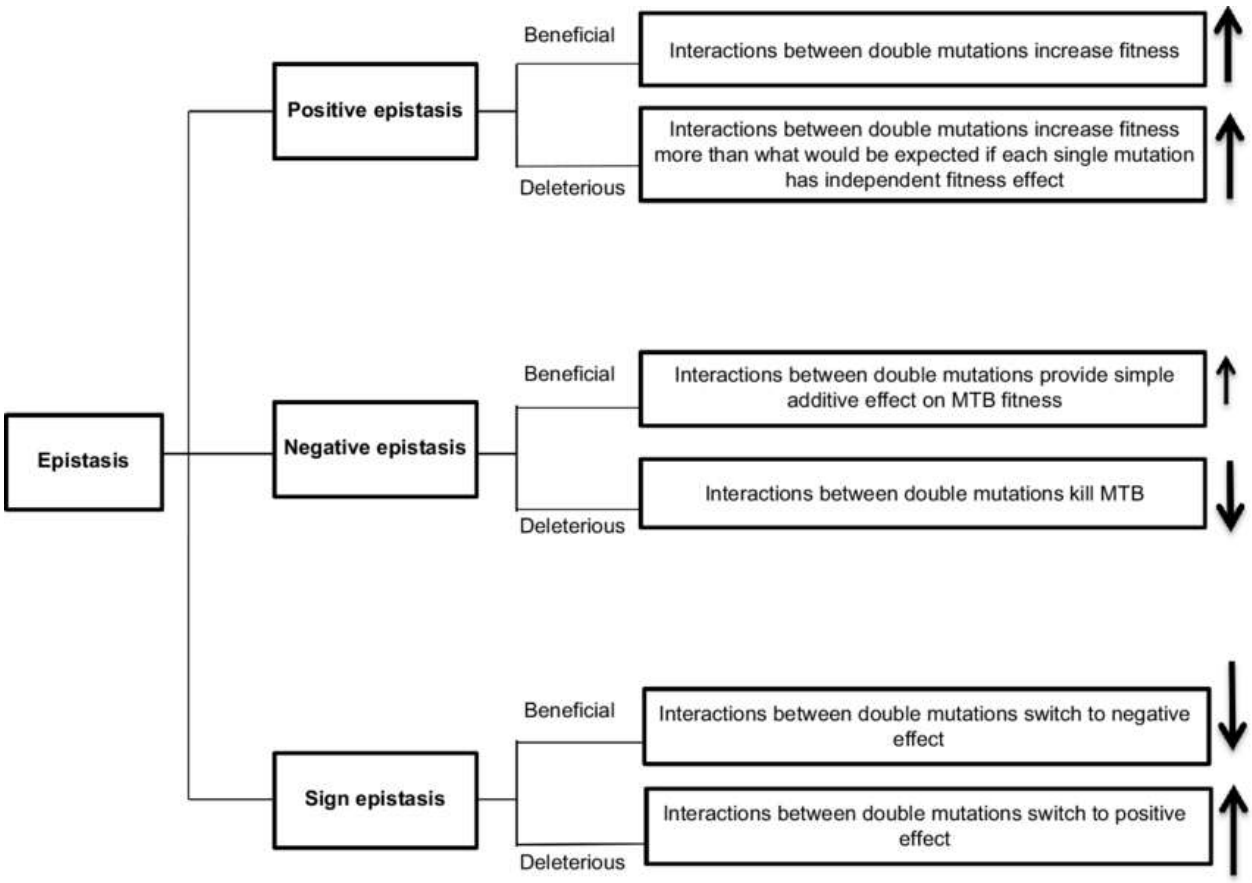

Figure 1. Forms of epistatic interaction between mutations [56]. (↑) indicated high MTB fitness and (↓) indicated low MTB fitness

Concluding remarks

TB is one of the deadly diseases that have led to increase morbidity and mortality through the world, after the initiation of drugs, which lead to great effect in reducing the devastating effects of this infection, especially used of the first-line drugs, but unfortunately, another major problem appeared in recent years that scientists and doctors faced which is drug resistant TB. It is a serious and growing problem in modern medicine and it is emerging as an outstanding public health threat particularly to first line drug, which is used for the treatment of new diagnosis patients. Many genetic and phenotypic mechanisms involved in development of resistance to therapy by *Mtb*. Understanding of these mechanisms allow scientists to initiate effective drug.

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Blood Cadmium Level and Its Association with Depressive Symptoms in a Sample of Iraqi Workers

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Abstract

Background The etiologies of mental disorders involve interactions among genetic, developmental, social, and environmental risk factors. An association between blood cadmium and depression was recently reported in young adults.

Objective To determine the prevalence of elevated blood cadmium level and to evaluate its association with depressive symptoms among adult Iraqi workers.

Methods A comparative cross-sectional study that conducted in two industries in Baghdad. It included 200 young adult male and female workers in the batteries factory (100 participants) who were exposed directly or indirectly to cadmium and in the textile factory (100 participants) unrelated to direct cadmium exposure. Blood sample was taken for measurement of blood cadmium level. Patient Health Questionnaire-9 was used to measure the symptoms of depression.

Results Mean of blood cadmium level was significantly higher in participants who had depressive symptoms than that in those who didn't have; in those with moderate to severe symptoms than that in those who had mild symptoms. Blood cadmium >0.21 µg/dl is predictive for risk of development of depressive symptoms. Statistically significant weak positive correlations were detected between blood Cd with total PHQ9 score, no. of cigarettes/day, duration of work in batteries factory and in textile factory.

Conclusion Elevation of blood cadmium level among sample of adult Iraqi workers especially in batteries factory is a significant health problem. Cadmium neurotoxicity may be a contributing factor for adverse mental health outcomes, even at levels generally considered to pose low or no risk.

Keywords Depression, mental illness, health, cadmium, workers, Iraq

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List of abbreviations: Cd = Cadmium, DSM-IV-TR = Diagnostic and Statistical Manual IV Text Revision

Introduction

Cadmium (Cd) is a heavy metal that is produced during the smelting of other metals, such as zinc, lead and copper. It is most frequently used in the manufacture of nickel-cadmium rechargeable batteries found in mobile phones and cordless equipment. It is also used in metal plating, some paints, plastics and fertilizers, and is found in cigarette smoke. Exposure to Cd occurs mostly in the workplace where Cd products are made. The general population can be exposed to Cd from cigarette smoke or eating Cd-contaminated foods [(1)]. Cd disrupt mitochondrial functions through many processes leading to energy metabolism and malfunctions in mitochondrial biochemical cascade, as suggested by several

studies on the pathophysiology of bipolar disorder, major depressive disorder, and schizophrenia. There is a correlation between mitochondrial dysfunction and psychiatric disorders [2]. Depression, anxiety, and stress are now the major mental health problems that cause disability globally, and no one is immune to these problems [3].

Depression manifests as loss of interest or pleasure, sadness, feelings of guilt or low self-worth, disturbed sleep or appetite, extreme tiredness, and poor concentration [4]. Globally, depression is ranked the 11th leading cause of Disability Adjusted Life Years worldwide, but, interestingly, the 3rd in the Middle East region [5]. Depression may lead to higher risk of dementia, premature mortality [6].

The pathophysiology of neuropsychiatric disorder is poorly understood, although there is a notion that structural changes occur in the brain of patients with neuropsychiatric disorder. In the brain, Cd cause lesions including decrease in total cortical volume, white matter, enlargement of cerebroventricular system, changes in gray and white matter, and abnormal laminar organization [7]. Cd toxicity affects several organs including kidney, lung, liver, and brain; an association between blood Cd and depression was recently reported in young adults [8]. The underlying biological mechanism of how Cd may play a role in depression could potentially involve dysregulation of the hypothalamic-pituitary-adrenal axis. Cd can increase the permeability of the blood brain barrier, leading to intracellular Cd accumulation in the brain in adult rats. Furthermore, Cd may contribute by perturbing the catecholamine/serotonin system; decreased levels of serotonin, dopamine, and norepinephrine in the brain have been found in adult male rats exposed to Cd [9].

There are no prior efforts done in Iraq to determine association of depressive symptoms with environmental exposure to Cd; so the aim of this study is to determine the prevalence of elevated blood Cd level and to evaluate its association with depressive symptoms among adult Iraqi workers.

Methods

Study design, setting, and time

This was a comparative cross-sectional study with analytic component that conducted in two industries in Baghdad (Batteries factory in Al-Waziriya and textile factory in Al-Kadhimiya) during a period of 18 months from 1st of Apr. 2020 to 1st of Oct. 2021.

Study population and sample size

The study population included 200 young adult male and female workers in the batteries factory (100 participants) who were exposed directly or indirectly to Cd according to their duties and in the textile factory (100 participants) unrelated to direct Cd exposure. They informed about the purpose of the study and those who agreed to participate were given an informed consent and enrolled in the study. Exclusion criteria included participants who had a recent history of psychological trauma ((Diagnostic and Statistical Manual IV Text Revision (DSM-IV-TR) that defines trauma as a direct personal experience of an event that involves actual or threatened death or serious injury) [10].

Data collection tools

Two different types of questionnaires were applied to all enrolled participants to collect needed information. First questionnaire was included questions to gather the following information: Age and gender, marital status, no. of children and family number, residence, educational level, type of job in the factory (manufacturing or office job), duration and weekly hours of working, smoking (cigarettes and shisha), alcohol drinking, and Blood Cd level: From each participant, blood sample, which was obtained from the antecubital area was sent to the Toxicology Center in Baghdad / Medical City for measurement of blood Cd level (normal value of Blood Cd is ≤0.3 µg/dl). The second questionnaire was a Patient Health Questionnaire-9 (PHQ-9), which is a validated depression screening tool to measure the

symptoms of depression including nine items. Each question would be pointed from 0 to 3, and the total score would be ranged from 0 to 27. Depression severity was characterized as none (0–4), mild (5–9), moderate (10–14), moderately severe (15–19), and severe (≥ 20) [11].

Statistical analysis

The data analyzed using Statistical Package for Social Sciences version 26. The data presented as mean, standard deviation and ranges. Categorical data presented by frequencies and percentages. Normality of data was tested by skewness value and showed that it is normally distributed. Independent t-test (two tailed) was used to compare blood Cd level accordingly. Receiver operating characteristic curve analysis was constructed for blood Cd as a predictor for risk of development of depressive symptoms. Pearson's correlation test was used to assess correlation between blood Cd level and certain variables. A level of P-value <0.05 was considered significant.

Results

In this study, mean of age was 31.58±6.42 years; 62.5% were males; 51.5% were married, 43% were finished higher education, 39% were current smokers, 53.8% were smoking ≤20 cigarettes/day, 45.5% were working in the selected factories for period <5 years and 61% of batteries factory workers were working in production sector. Regarding depressive symptoms, 35% of study participants showed features of moderate level of depressive symptoms; while 29% of them didn't show significant features of depressive symptoms. Cd level was high in 35.5% of study participants (Table 1).

Mean of blood Cd level was significantly higher (P <0.05) in participants who had depressive symptoms than that in those who didn't have; in those with moderate to severe symptoms than that in those who had mild symptoms; in participants who are working in batteries factory than that who are working in textile factory, in those who are working in production sector than that in those who are working in administration, and in smokers than that in nonsmokers as shown in table (2).

Receiver operating characteristic (ROC) curve analysis was constructed for blood Cd as a predictor for risk of development of depressive symptoms. The cut point of blood Cd was 0.21 µg/dl. So, blood Cd >0.21 µg/dl is predictive for risk of development of depressive symptoms, as a large significant area under the curve (AUC=60%) indicating significant association between higher level of blood Cd with risk of development of depressive symptoms. Blood Cd level was 66.2% sensitive, 56.9% specific, and 63.5% accurate in predicting risk of development of depressive symptoms (Table 3) and (Figure 1).

Statistically significant weak positive correlations were detected between blood Cd with total PHQ9 score (r=0.335, P=0.001), no. of cigarettes/day (r=0.301, P=0.007), duration of work in batteries factory (r=0.357, P=0.001) and in textile factory (r= 0.214, P= 0.032) (Table 4).

Table 1. Distribution of study participants by certain characteristics

Variable		No. (n= 200)	Percentage (%)
Age (Year)	<30	82	41.0
	≥30	118	59.0
Gender	Male	125	62.5
	Female	75	37.5
Marital status	Single	52	26
	Married	103	51.5
	Divorced	21	10.5
	Widowed	24	12.0
Educational level	Illiterate	14	7.0
	Primary school	45	22.5
	Secondary school	55	27.5
	Higher education	86	43.0
Smoking Status	Current smoker	78	39.0
	Ex-smoker	9	4.5
	Nonsmoker	113	56.5
Number of cigarette smoking/day; n=78	≤ 20	42	53.8
	> 20	36	46.2
Type of job (Batteries **factory);** n=100	Production sector	61	61.0
	Administration job	39	39.0
Duration of work (Year)	< 5	91	45.5
	5 - 9	62	31.0
	≥ 10	47	43.5
Depressive symptoms	No	58	29.0
	Mild	64	32.0
	Moderate	70	35.0
	Severe	8	4.0
Cd level	High	71	35.5
	Normal	129	64.5

Table 2. Comparison in blood Cd level by certain characteristics

Variable		Blood Cd (µg/dl)	P - value
Depressive symptoms	Yes	0.272 ± 0.1	0.038
	No	0.237 ± 0.1	
Severity of depressive symptoms	Mild	0.22 ± 0.08	0.001
	Moderate to severe	0.3 ± 0.1	
Factory type	Batteries	0.32 ± 0.08	0.001
	Textile	0.2 ± 0.08	
Type of job in batteries	Production sector	0.34 ± 0.07	0.001
	Administration job	0.28 ± 0.09	
Smoking Status	Current smoker	0.29 ± 0.11	0.004
	Nonsmoker	0.24 ± 0.09	

Table 3. Diagnostic accuracy of blood cadmium for prediction of risk for development of depressive symptoms

Cd (µg/dl)	Cut-off value	Sensitivity	Specificity	PPV	NPV	Accuracy
	0.21	66.2%	56.9%	79%	40.7%	63.5%

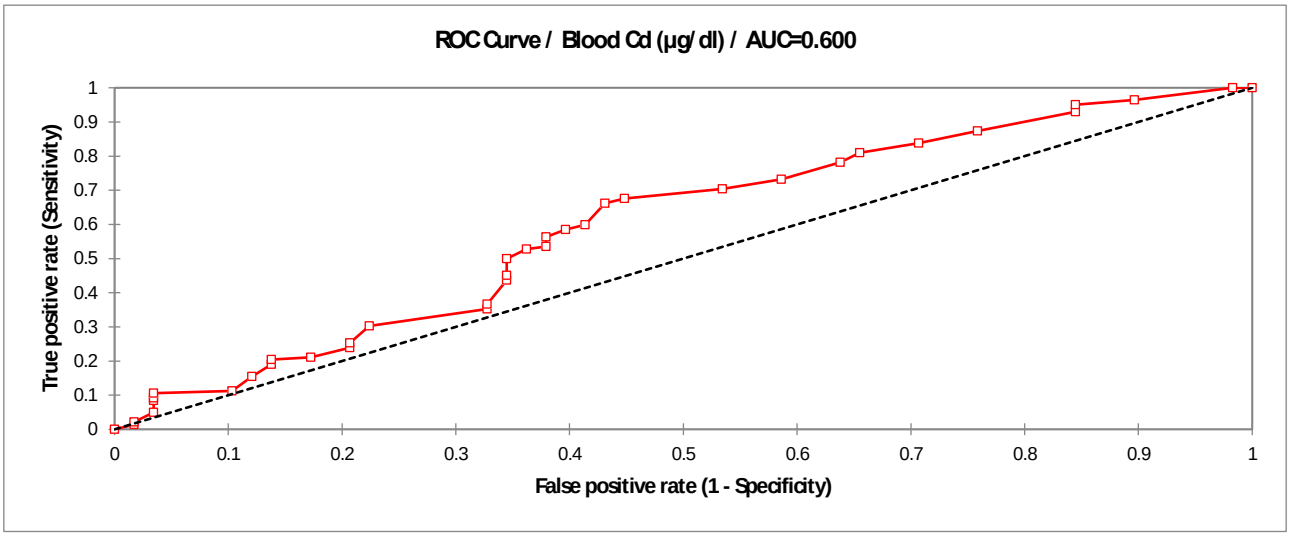

Figure 1: ROC curve for blood cadmium level in predicting risk of development of depressive symptoms

Table 4. Correlation between blood cadmium and certain parameters

Variable		Blood Cd level (µg/ml)	
		r	P - value
Total PHQ9 score		0.335	0.001
No. of cigarettes/day		0.301	0.007
Duration of work (Year)	Batteries factory	0.357	0.001
	Textile factory	0.214	0.032

Discussion

Improving mental health contributes to promoting healthy development and achieving educational, social, and economic goals, and avoiding communicable and non-communicable conditions [12]. In the current study, a high level of Cd was significantly observed in workers of batteries factory more than in those of textile factory. This finding is in accordance with a study conducted by Baloch et al. in Pakistan 2020 when they noticed that mean value of Cd concentration in blood samples of workers of both workshops (batteries recycling factoring and welding workers) were five to eight folds elevated as observed for referent adolescents [13]. This study also revealed that depressive symptoms were more obvious in those had a significantly higher means of blood Cd levels than those who didn't have. Agreements observed in studies conducted by Buser et al. in 2017 [9], by Kostrubiak et al. in 2017 [14], and by Berk et al. in 2014 [15].

The biological plausibility of Cd effect on depression is unclear because of the scarcity of studies pertaining to Cd exposure and neurobehavioral outcomes. Studies in animals have shown that Cd can increase the permeability of the blood brain barrier, which can cause an intracellular Cd accumulation in the brain and cell dysfunction in adult rats [16]. Impairment in the mono-aminergic neurotransmission system is associated with the depression and anxiety disorder, and Cd may contribute to the etiology of depression through perturbation of the catecholamine/serotonin system. Adult male rats exposed to Cd show a decreased content of serotonin, dopamine and norepinephrine in all brain regions [17]. Other experimental studies on rats indicate that early exposure to Cd can induce behavioral and neurotoxic effects, including a decrease of locomotor activity or an increase of anxiety-like behavior, which reported an impaired cognition and enhanced anxiety-like behavior related to high acetylcholinesterase activity and a decrease of Na^+, K^+-ATPase activity in pubertal male rats treated with cadmium in the diet [18]. Moreover, present study showed a significantly higher mean of blood Cd in current smokers than that in nonsmokers. Smoking is an exogenous source of metals contamination in human body; a single cigarette contains 1.0-4.5 µg Cd and at least one tenth of the metal content of a cigarette is inhaled [19].

Cadmium toxicity affects several organs including kidney, lung, liver, and brain; an association between blood Cd and depressive symptoms was recently reported in young adults [8]. Results observed in Kostrubiak et al. study in 2017 agreed to the current one in that exposure in patients with depressive symptom were predominately current smokers [14]. Another similar result was published in Scinicariello et al. study in 2015, in which claimed that smoking status was significantly associated with depressive symptoms in those exposed to Cd [8]. Since Cd exposure is associated with depression, continued efforts at reducing Cd population exposures mainly via tobacco smoking cessation programs, which have the added benefit of decreased Cd exposure through second- and third-hand smoke, may help decrease the population incidence of depression [20]. There is a strong association between smoking and depression; however, this link may be bidirectional, depression increases the risks of smoking, and smoking increases the risks of depression [21].

In conclusion, elevation of blood Cd level among adult Iraqi workers especially in batteries factory is significant health problem and rises in production sites with increased duration of work that disrupts individuals' well-being, have predictive effects on the development of depressive symptoms and can affect the severity of these symptoms. These findings suggest that Cd neurotoxicity may be a contributing factor for adverse mental health outcomes, even at levels generally considered to pose low or no risk.

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Author contribution

Dr. Al-Samaraee: Designing the work, collecting part of data, analysis and interpretation of data, and drafting the work. Took public responsibility for suitable portions of the content after participating sufficiently in the work. Dr. Sahib: Designing the work, collecting part of data, interpretation of data, and final approval of the version. Took public responsibility for suitable portions of the content after participating sufficiently in the task.

Conflict of interest

None.

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Risk Factors of Acne Vulgaris among Mosul University Students from Iraq

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Abstract

Background Acne vulgaris is a common skin disease, which is a significant health problem among adolescents and young adults. It affects 85-100% of people at some point in their lives, and it usually begins at puberty. Acne can persist into the 30s and beyond.

Objective To evaluate the risk factors for development of acne vulgaris among Mosul University students in Mosul- Iraq.

Methods A case-control study conducted to 300 persons (150 cases and 150 controls) aged 18-35 years selected randomly among Mosul University students who attended university primary health care that located inside Mosul University, Iraq, during the sixth month period from august 2019 to January 2020.

Results Of the acne vulgaris cases (150 cases) that included in the study, 45 % are males and 55% are females, those with age group 18-23 years has 1.8 risk for developing of acne, always stress has 2.7 risk, female cases with irregular cycle have 58 times risk. 53% of female & 46% of male have acne on their face and male patients have 35 risk for acne on their shoulder, oily skin has 3 times risk, two and more members in family with acne have 4 risk followed by 3 times risk of one family member with acne, student's mobile use has about 2 times risk for developing of acne.

Conclusion There were several significant factors associated with acne formation in the study, which play a role in acne formation including age, psychological status, menstrual cycle irregularity, site of appearance of acne, nature of the skin, family history, using student's mobile.

Keywords Acne vulgaris, risk factors, university students

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List of abbreviations: BMI = Body mass index, DHEA = Dehydroepiandrosterone, GAGS = Global acne grading system, PCOS = Poly cystic ovary syndrome

Introduction

Acne vulgaris is an inflammatory disorder of the pilosebaceous unit, which runs a chronic course and can lead considerable physical and psychological problems if diagnosed or treated properly. Acne vulgaris is triggered by *Cutibacterium acnes* in adolescence, under the influence of normal circulating dehydroepiandrosterone (DHEA). It is a very common skin disorder, which can present with inflammatory and non-inflammatory lesions chiefly on the face but can also occur on the upper arms, trunk, and back [1-3]. Several exacerbating factors have been suggested including diet, menstruation, sweating, personal stress, ultraviolet radiation, application of pomades and occupation [4], use of medications like lithium, steroids, and anticonvulsants, exposure to excess sunlight, use of occlusive wear like shoulder pads, headbands backpacks, and underwire brassieres, endocrine disorders like polycystic

ovary syndrome and even pregnancy have also reported [5]. The association between diet and acne can no longer be dismissed. Compelling evidence shows that high glycemic load diets may exacerbate acne (also, low glycemic load diet that resulted in the improvement of acne lesions) [6,7]. Food with a high glycemic index is rapidly absorbed, increases serum glucose levels and stimulates increased glucose-dependent insulin signaling [8]. Acne vulgaris affects 85% of adolescents, often starts in preadolescence, and persists into adulthood [9]. Acne lesions may vary in number during the natural course of the disease and multiple measurements have been developed, which is based on clinical examination and photographic documentation, to measure the clinical severity. The grading of acne based on the type of lesions, affected surface area and their severity that can help in deciding which therapies are needed in each individual. However, no grading system has been accepted universally. The Global Acne Grading System (GAGS) is a quantitative scoring system to assess acne severity. It was first developed by Doshi and colleagues in 1997 [10]. The total severity score is derived from summation of six regional sub scores. Each is derived by multiplying the factors: 2 for forehead, 2 for each cheek, 1 for nose, 1 for chin, 3 for both chest and back by the most heavily weighted lesion within each region. The regional factors were derived from consideration of surface area and distribution and density of pilosebaceous units, according to this score acne was graded as mild, moderate, severe and very severe, as showing in the following table 1 [11,12].

Table 1. The Global Acne Grading System (GAGS) [10]

Location	Factor X Grade (0-4) = Local score	Global score
Forehead	2	0 = None 1-18 = Mild 19-30 = Moderate 31-38 = Severe > 39 = Very severe
Right cheek	2	
Left cheek	2	
Nose	1	
Chin	1	
Chest & upper back	3	

Grade 0: No lesions; 1 ≥ One comedone; 2 ≥ One papule; 3 ≥ One pustule; 4 ≥ One nodule

Topical therapy is the first-line choice for mild to moderate acne and important adjuvant treatment for moderate to severe acne that is being treated systemically [13].

This study aimed to assess the risk factors that might play essential role in the occurrence of acne vulgaris in university students in Mosul, Iraq.

Methods

Settings and study design

This case-control study assessed factors associated with the development of acne, which selected by systematic randomization as every other one of university students that visit the University Primary Health Care that located inside Mosul University aged from 18-35 years (both under graduate and postgraduate university students). It is categorized as a case or control by clinical examination and included in the study during the sixth month period (from August 2019 till January 2020), 150 cases and 150 controls were included in the study.

Ethical consideration

All patients provided a verbal consent before participating in the study. The protocol was reviewed and approved by the Ethics Committee at the participating center.

Case and control definition

Cases: morning and evening under graduate and postgraduate university students aged (18-35) years (including both males and females) were diagnosed with acne vulgaris of any grade (ranging from mild to severe), as assessed by clinical examination during the visit.

Controls: morning and evening under graduate and post graduate university students aged (18-35) years (including males and females) that attend University Primary Health Care for conditions other than acne and who were not diagnosed with acne during the visit.

Retrieving data

The main source of data was obtained directly from the cases and controls by the investigator through direct interview with the patients, from their case sheets of each case or control and filling the questionnaire form, which was prepared to record all relevant information related to cases and controls in the study sample.

Procedure

A structured questionnaire was administered during their visits and was developed to collect general sociodemographic information, personal habits, smoking, anthropometric measurements, menstrual pattern and relation of acne with menstrual cycle, living with family, washing face per day, washing body per week, season do acne appear, location of acne, skin type, family history of acne vulgaris, and a food frequency questionnaire. Also, we assess if there is any friction or pressure on the skin by person's mobile or helmets [14-21]. After preparing it, it was reviewed by other dermatological doctor and it is used in English and Arabic).

Analysis of data

Data were collected based on the frequencies of occurrence and statically analyzed with a Pearson's Chi-square test using (SYSTAT 12) statistical software to assign significant differences between the groups where the significance level was set at $P<0.05$. The effects of identified factors were presented as odd ratio, p-value, with 95% confidence interval.

Results

Table 2 shows that of 150 acne cases, 45% are males and 55% are females with p-value 0.106 with no statistically association with acne between cases and controls, 53.3% of cases between age group (18-23) with p-value <0.05 with significant association, 52.4% of acne cases are single with no statistical association, 49.2 of acne patients living with family, 64.2% of acne cases have always stress with statistically strongly significant association with acne with 2.7 risk, the frequency of washing face or body has no statistically association with acne, of acne female patients menstrual cycle irregularity has 58 times risk with statistical strongly association with acne, 100% of acne female cases have acne before or during menstruation.

Table 3 reveals that of acne patients 46.6% of male and 53% of female have acne on their face and having acne on shoulders have statistically association with acne with 35 risk between male and female, 43.2% of male and 56.8% of female have acne in summer season but have no statistically association with acne.

Table 4 shows 47.5% of cases have normal body mass index has no statistical association with acne, oily skin has 3 times risk for acne with statistically strongly significant association with acne, dry skin has protective factor, face complexion has no statistical association with acne , two and more members in family with acne have 4 risk for acne followed by 3 times risk of one family member with acne with statistical association of acne, cigarette smoking has no statistically association with acne.

Table 5 reveals that dietary intake have no statistically significant association with acne.

Table 6 demonstrates that person's mobile use has about 2 times risk for developing of acne with statistically association with acne.

Table 2. Sociodemographic, psychological, self-hygiene, menstrual regularity factors associated with acne formation (n=300)

Characteristics		Case		Control		X_2	P-Value	Odd ratio	95% of C.I.
		n	%	n	%				
Sex	Male	70	45.46	84	54.54	2.615	0.106	0.688	0.436-1.083
	Female	80	54.79	66	45.21				
Age	18-23	119	53.6	103	46.3			1.752	1.037-2.960
	24-30	29	43.9	37	56	7.456	0.024	0.732	0.422-1.268
	31-35	2	16.6	10	83.3			0.189	0.041-0.879
Marital status	Single	128	52.4	116	47.5	3.162	0.075	1.705	0.943-3.083
	Married	22	39.2	34	60.7				
Living with family	Yes	136	49.2	140	50.7	0.725	0.395	0.694	0.298-1.616
	No	14	58.3	10	41.6				
Stress	Always	81	64.2	45	35.7			2.739	1.704-4.402
	Absence	22	30.1	51	69.8	22.443	0.000	0.334	0.190-0.587
	Occasional	47	46.5	54	53.4			0.811	0.502-1.311
Washing face/day	1-2	37	53.6	32	46.3			1.207	0.704-2.070
	3-5	99	48.2	106	51.7	0.958	0.619	0.806	0.495-1.312
	>5	14	56	11	44			1.301	0.570-2.966
Washing body/ day	1-2	19	47.5	21	52.5			0.891	0.458-1.735
	3-5	101	48.7	106	51.2	0.910	0.635	0.856	0.524-1.396
	>5	30	56.6	23	43.3			1.380	0.759-2.510
Menstrual irregularity (Female)	Yes	38	97.4	1	2.6	39.063	0.000	58.810	7.777-444.72
	No	42	39.2	65	60.7				
Acne in relation to menstruation (Female)	Yes	13	100	0	0	11.773	0.001	1.985	1.677-2.350
	No	67	50.3	66	49.6				

Table 3. Comparison of cases between males and females regarding location of acne and season do acne appear (n=150)

Characteristics		Acne positive				P-Value	Odd ratio	95% of C.I.
		Male		Female				
		n	%	n	%			
Location of acne Case only	Face	70	46.67	80	53.33	0.380	1.313	0.715-2.411
	Shoulders	22	38.5	35	61.4	0.000	35.139	4.636-266.4
	Others	2	66.6	1	33.3	0.448	2.473	0.221-27.7
Season do acne appear	Summer	54	43.2	71	56.8	0.630	0.862	0.472-1.574
	Winter	11	61.0	7	38.8	0.135	2.110	0.802-5.534
	Autumn	14	42.4	19	57.6	0.797	0.905	0.428-1.915
	Spring	5	38.4	8	61.6	0.653	0.767	0.254-2.327

Table 4. Acne rate regarding body mass index, skin type, family history of acne vulgaris, cigarette smoking (n=300)

Characteristics		Case		Control		Odd ratio	P-Value	95% of C.I.
		n	%	n	%			
BMI	Under weight	11	50	11	50	1	1.000	0.429-2.334
	Normal	95	47.5	105	52.5	0.740	0.221	0.458-1.197
	Over weight	30	58.8	21	41	1.536	0.167	0.838-2.812
	Obese class I	12	52	11	47.8	1.099	0.828	0.478-2.525
	Obese class II	1	50	1	50	1	1.000	0.103-9.665
	Obese class III	1	50	1	50	1	1.000	0.103-9.665
Skin type	Dry	12	24	38	76	0.256	0.000	0.129-0.509
	Oily	99	64.2	55	35.7	3.353	0.000	2.090-5.379
	Mixed	39	40.6	57	59.3	0.573	0.026	0.351-0.936
Face complexion	Fair	1	33.3	2	66.6	0.497	0.562	0.064-3.841
	Moderate	144	49.6	146	50.3	0.658	0.520	0.195-2.220
	Dark	5	71.4	2	28.5	2.552	0.251	0.560-11.57
Family history of acne vagaries	Nil	70	37.4	117	62.5	0.247	0.000	0.150-0.407
	1 Family member	55	67.9	26	32	2.761	0.000	1.618-4.711
	2 More member	25	78	7	21.8	4.086	0.001	1.744-9.547
Cigarette smoking	Never	113	50.2	112	49.7	1.036	0.894	0.616-1.743
	Sometimes	18	54.5	15	45.5	1.227	0.580	0.600-2.508
	Daily	19	45.2	23	54.8	0.801	0.506	0.419-1.530

Table 5. Comparison of dietary intake frequency between cases and control (n=300)

Diet		Case		Control		Chi	P-Value	Odd ratio	95% of C.I.
		n	%	n	%				
Chocolate	Often	108	50.7	105	49.2	0.146	0.703	1.102	0.670-1.811
	Seldom	42	48.2	45	51.7				
Sweets	Often	108	50.4	106	49.5	0.065	0.798	1.067	0.648-1.757
	Seldom	42	48.8	44	51				
Potato ships	Often	106	53	93	46.7	2.523	0.112	1.477	0.913-2.387
	Seldom	44	43.5	57	56.4				
Ice-cream	Often	106	51.7	99	48.2	0.755	0.385	1.241	0.763-2.017
	Seldom	44	46.3	51	53.6				
Carbonated drink	Often	111	52.8	99	47	2.286	0.131	1.466	0.893-2.406
	Seldom	39	43	51	56.6				
Milk	Often	79	45.6	94	54.3	3.072	0.080	0.663	0.419-1.050
	Seldom	71	55.9	56	44				

Table 6. Comparison of acne between case and control based on Friction or pressure on skin of the face (n=300)

Friction or Pressure on the skin by		Case		Control		Chi	P-Value	Odd ratio	95% of C.I.
		n	%	N	%				
Mobile	Yes	48	61.5	30	38.4	5.613*	0.018	1.882	1.114-3.179
	No	102	45.9	120	54				
Helmits	Yes	9	50	9	50	0.000	1.000	1	0.397-2.521
	No	141	50	141	50				

Discussion

From this study, it has been revealed that of acne cases 45% are male and approximately 55% are female with no statistically association with acne between cases and controls, this result is similar to the study done by Yassin and Mohammed in 2020 in Baghdad, Iraq [22]. As with men, female acne is the result of too much oil being produced by the skin which is the result in clogged pores [5]. Age group has statistically association with acne with age group 18-23 years with approximately 2 times risk, as this nature is considered a normal aspect of the maturation process, this result is similar to the result study in Bangladesh 2019 by Ettel [23].

Marital status has no statistical association with acne, this result is similar study done in India 2017 by Qidwai et al. [18]. Living with family has no statistical association with acne as in the study done in Syria in 2014 by Al-kubaisy et al. [16].

Having always stress has 3 times risk for acne, we confirmed the association between acne prevalence and degree of stress in which we found that students who always were subjected to continuous stress demonstrated higher rate of acne, most probably, this relation could be attributed to the increased production of cortisol during emotional stress which in turn increased the sebum production [15], this result is similar to study in Damascus, Syria 2014 by Al-kubaisy. [16].

There is no statistically significant association with washing face or body per day as a risk factor for acne, this result is similar to study done in south India 2015 by Durai and Nair [17].

Irregular cycle for female cases has a 58 times risk with statistically significant association with acne, this result is similar to the study done in Syria in 2014 by Al-kubaisy et al. [16], which is stated that hyperandrogenicity during the menstrual cycle especially if its irregular may have contributed to the acne formation.

Face is the commonest site of acne followed by shoulders which has statistical association with acne this result is similar to the study done in 2015 in India by Durai and Nair ([17], as oil glands are all over the body, but those are the places where there are the most. In both males and females, acne mostly appear during summer as a study done in India 2017 by Qidwai et al. as in summer there increase in sweating and sebum production [18].

Majority of acne cases had normal body mass index, the association between body mass index and acne is not significant although there were proportion who were underweight, normal, overweight or obese in almost all the grades of acne, this is similar to study done in Nepal 2018 by Neupan et al. [19].

Skin type has statistical association with acne with oily skin has 3 times risk for acne as sebum overproduction is the result of excessive androgen hormones or a heightened sebaceous gland sensitivity to normal levels of androgen hormones, this result is similar to study done in Louisiana 2019 by Oge' et al. [24].

Medium face complexion has 49.6% of the cases as oily skin and medium complexion are more likely to be predisposed to skin damage than light and dark complexion, dry skin is the next leading skin condition prone to acne, this result is similar to the study in north central India in 2017 by Qidwai et al. [18].

Family history of acne vulgaris has statistically significant association with acne with 4 times risk for 2 and more family member with acne and 2 times risk for one family member with acne. It is further supported by a large-scale twin genetic modeling study; which was conducted in the United Kingdom. It was evident that 81% of acne variances were due to genetic factors; while unshared environmental factors made up the remaining 19%, this study is similar to the study done in 2012 in journal American academy by Di Landro et al. [25].

Cigarette smoking has no statistically significant association with acne, like the study done in Malaysian 2018 by Suppiah et al. [26].

Dietary intake has no statistically association with acne, a concise systematic review by Magin et al. [27] of seven studies, including one randomized controlled trial, concluded that there was no clear, positive evidence that any dietary components increase acne risk as in the study done in India in 2017 by Qidwai et al. [18].

Friction or pressure on the skin by person's mobile has approximately 2 times risk for acne like study done in America 2019 by Torjesen [28] as acne mechanica is defined as being any acneiform eruption in areas of friction, pressure, stretching, rubbing, pinching or occlusion of the skin in any individual, regardless of pre-existing acne. It presents as inflammatory papules and pustules that can progress to nodules and cysts.

In conclusions, acne is a health and psychological problem among university students which is highly related to age of youth. There were several significant factors associated with acne formation in the study which were age, stress, menstrual cycle irregularity, location of acne, skin type, family history of acne vulgaris and person's mobile use.

The recommendations of the current study is to minimize and decrease the prevalence and the aggravating factors of acne vulgaris as much as possible among the medical students of Mosul University Students in Mosul, Iraq to get more beneficial quality of the life and promotions of the psychological future as we can prevent its complications on the skin.

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Conflict of interest

None.

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Neonatal Outcomes in Gestational Diabetes Mellitus

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Abstract

Background Gestational diabetes mellitus (GDM) is a common and serious maternal complication, in which hyperglycemia develops at any time during pregnancy due to progressive insulin resistance. It affects about 14% of pregnancies worldwide. There are many adverse effects of GDM that compromise the fetus and neonate.

Objective To compare neonatal outcomes according to type of treatment for GDM.

Methods A prospective study conducted at the Department of Pediatrics, (Neonatal Intensive Care Unit; NICU) and Obstetric in Al-Imamein Al-Kadhimein Medical City in Baghdad during a period from 1st of march 2019 to 1st of January 2020. The study included 100 neonates delivered by mothers with GDM, divided in to four groups according to their mothers' therapy; (diet group: 18 neonates, metformin group: 36, insulin group: 26, mixed group: 20).

Results Neonates in metformin group had a higher chance of having normal birth weight comparing with others, but neonates in insulin group have higher percent of prematurity, macrosomia, large for gestational age or small for gestational age, hypoglycemia and jaundice among others. No significant statistical difference between metformin and insulin, in mode of delivery, Apgar score, respiratory distress syndrome, hypocalcaemia, anomalies, and NICU admission but can occur more in insulin group.

Conclusion Metformin was able to reduce the risk of neonatal complications, therefore, it can be a good alternative for insulin in the treatment of GDM.

Keywords Gestational diabetes mellitus, macrosomia, metformin, insulin

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List of abbreviations: ANC = Antenatal care, CHD = Congenital heart disease, GDM = Gestational diabetes mellitus, HR = Heart rate, IDF = International diabetes federation, LGA = Large for gestational age, NICU = Neonatal intensive care unit, OFC = Occipital frontal circumference, PCV = Packed cell volume, RR = Respiratory rate, RDS = Respiratory distress syndrome, SGA = Small for gestational age

Introduction

Gestational diabetes mellitus (GDM) is one of the most common and serious conception complications, in which spontaneous hyperglycemia develops at any time during pregnancy [(1)]. According to the most recent (2017) International Diabetes Federation (IDF) estimates, GDM affects approximately 14% of pregnancies worldwide, representing approximately 18 million births annually [(2)]. Risk factors include overweight/obesity, westernized diet and micronutrient deficiencies, advanced maternal age, a family history of insulin resistance and/or diabetes, and ethnicity among women [(3)]. Pregnancy itself is characterized by insulin resistance [(4)]; placental production of diabetogenic hormones such as human placental lactogen in late pregnancy, leading to

progressive insulin resistance; when adaptation of β-cell hyper functionality during pregnancy fails to compensate maternal insulin resistance, GDM develops [5,6]. In utero, exposure to maternal hyperglycemia increases the incidence of perinatal complications. There are many adverse effects of GDM that compromise the fetus including, fetal anomalies or intrauterine death, macrosomia, birth injuries and asphyxia, respiratory distress syndrome, metabolic disorders, growth imbalance, hypoglycemia, hyperbilirubinemia, polycythemia, hypocalcaemia, and some long-term complications. More significantly, GDM places the offspring at risk of insulin resistance and type 2 diabetes mellitus, obesity and cardiovascular disease in adulthood [7-9]. Considering adverse effects mentioned, GDM treatment seems to have great importance and benefits [10]. Medical treatment is initiated if glucose control levels are not achieved by lifestyle modifications such as exercise and dietary changes [11]. Traditionally, insulin has been the golden key for treatment in GDM patients. No placental passage and fine glucose level control, are established benefits of insulin administration in pregnancy [12]. On the other hand, insulin usage has some disadvantages and doubts remain about insulin consumption inconveniences in pregnancy. These include the need for multiple injections, maternal hypoglycemic risk, higher maternal weight gain during pregnancy (possibly due to increased appetite), and increased treatment cost [13]. Various studies have shown that an oral anti glycemic drug may not only have better maternal and fetal consequences but also could bring patients' acceptance [14]. Metformin has been introduced as an alternative drug for insulin in GDM treatment theoretically [15]. This agent induces less gluconeogenesis and higher peripheral glucose uptake. Reducing insulin resistance is of great concern as well many studies approved that metformin did not induce maternal hypoglycemia, excessive maternal weight gain during pregnancy, and major fetal anomalies [16]. In some other studies, it has been shown that metformin administration in GDM has not been accompanying neonatal disorders [4,17].

This study aimed to compare neonatal outcomes according to type of treatment for GDM.

Methods

A cross-sectional study conducted at Department of Pediatric and Obstetric in Al- Al-Imamein Al-Kadhimein Medical City in Baghdad during a period from 1st of March 2019 to 1st of January 2020. The study included 100 neonates delivered by mothers with GDM, the mother's age between 18-45 years old, who had been already diagnosed by obstetrician and gynecologist and on treatment with exclusion of mothers with type 1 and type 2 diabetes mellitus, other chronic diseases and with still birth. All mothers were selected from post-delivery ward in the hospital. Mother data was collected from the mothers themselves by direct questionnaire included the following: Mother age, last menstrual period (LMP), expected date of delivery (EDD), gravity, mode of delivery, type of therapy, antenatal care (ANC), their compliance with therapy, HbA1c was recorded in some of mothers. The evaluated neonatal outcomes data were collected by the neonatal intensive care unit (NICU) doctors or patients case sheets, soon post-delivery or during first week of life if admitted to NICU and included the following: Neonatal age, sex, birth weight, length, occipital frontal circumference (OFC), heart rate (HR), respiratory rate (RR), gestational age, weight in relation to gestational age, Apgar score at 1 and 5 minutes and any obvious congenital anomalies. After neonatal resuscitation, the blood glucose level of them checked during the first 2 hour after birth to detect hypoglycemia, specific investigations should be done according to the cause, such as packed cell volume (PCV) and total serum bilirubin in plethoric or jaundiced neonate, serum calcium if there is suspicion of hypocalcaemia, chest x-ray and echo study in respiratory distress syndrome (RDS) or

congenital heart disease (CHD). Finally, the neonates were divided in to four groups according to types of their mothers' therapy: diet group 18 neonates, metformin group 36, insulin group 26, and mixed group 20, and we evaluate their characteristic, complications and compare them among groups, according to their mother's treatment. All mothers were verbally informed about the study and they were asked permission to make their neonates being part of the study.

Statistical analysis

This is a cross-sectional study; data were presented as frequency and percentage. Comparison of variables between types of therapy of gestational diabetes using Fisher exact test, chi square and Yates chi square test were used. P value < 0.05 considered as level of significance. Statistical package for social sciences (SPSS) version 23 were used.

Results

The study sample consisted of 100 neonates of mothers diagnosed as gestational diabetes mellitus, and on treatment. After data collection they were grouped according to maternal therapy into four groups: 1. dietary therapy, 2. metformin, 3. insulin, 4. metformin and insulin. From a hundred mothers, eighteen mothers were treated with diet, thirty sex with metformin, twenty-six with insulin, and twenty with mixed (insulin and metformin). The neonatal outcomes were analyzed depend on the mother therapy used. The mean age of neonates was 2.01 days (SD±1.62).

Regarding sex of newborns, 53 (53%) of them were females, 47 (47%) were males, and 70 (70%) of them were delivered by cesarean section, 39 (39%) were preterm, 35 (35%) were macrocosmic babies, and 44 (44%) were of normal birth weight, and according to weight for gestational age chart, 45 (45%) of neonates were adequate for gestational age, 37 (37%) large for gestational age, and 18 (18%) small for gestational age.

Apgar score at five minutes was low in 54 (54%) of newborns and 78% of neonates need admission to NICU due to single or multiple complications and the remainder 22% were normal.

Diet and metformin treatment groups show higher rate of term delivery (72.2%) for both comparing to higher rate of prematurity in insulin treatment group (65.4%), also, there was significant difference in gestational age according to type of mother therapy (p value = 0.014) (Table 1).

Table 1. Comparison of gestational age according to maternal therapy

Parameter		Maternal therapy				P value*
		Insulin N=26	Mixed N=20	Diet N=18	Metformin N=36	
Gestational age	Preterm	17 (65.4%)	7 (35.0%)	5 (27.8%)	10 (27.8%)	0.014
	Term	9 (34.6%)	13 (65.0%)	13 (72.2%)	26 (72.2%)	
P value**	Insulin vs other groups		0.073	0.031	0.005	
	Mixed vs other groups			0.734	0.762	
	Diet vs metformin				1.000	

* Chi square test, ** Fisher exact test

Mothers who were treated with metformin had a higher chance of having babies with normal birth weight (55.6%) comparing with those treated with other therapies, but neonates in insulin group have higher percent of low birth weight (26.9%) and macrosomia (50%) among others (p value=0.038), which mean that there is a relation between neonatal birth weight and mothers' therapy (Table 2).

Table 2. Comparison of birth weight according to maternal therapy

Parameter		Maternal therapy				P value*
		Insulin N=26	Mixed N=20	Diet N=18	Metformin N=36	
Birth weight	Low	7 (26.9%)	5 (25.0%)	3 (16.7%)	6 (16.7%)	٠.٢٩٧
	Normal	6 (23.1%)	9 (45.0%)	9 (50.0%)	20 (55.6%)	
	Macrosomia	13 (50.0%)	6 (30.0%)	6 (33.3%)	10 (27.8%)	
P value*	Insulin vs other groups		0.249	0.179	0.038	
	Mixed vs other groups			0.820	0.184	
	Diet vs metformin				0.233	

* Chi square test

Regarding weight for gestational age, neonates who delivered to mothers treated with metformin have a lower chance of having SGA and also had slightly about twice the chance of having a baby AGA, comparing with insulin group which show higher percent of having LGA or SGA babies (p value=0.045) (Table 3**).**

Table 3. Comparison of weight for gestational age according to maternal therapy

Parameter		Maternal therapy				P value*
		Insulin N=26	Mixed N=20	Diet N=18	Metformin N=36	
Gestational age	Preterm	17 (65.4%)	7 (35.0%)	5 (27.8%)	10 (27.8%)	0.014
	Term	9 (34.6%)	13 (65.0%)	13 (72.2%)	26 (72.2%)	
P value**	Insulin vs other groups		0.073	0.031	0.005	
	Mixed vs other groups			0.734	0.762	
	Diet vs metformin				1.000	

* Chi square test, ** Fisher exact test, SGA=small for gestational age, AGA=adequate for gestational age, LGA=large for gestational age

The type of therapy did not significantly affect the mode of delivery in metformin and insulin groups, but in diet and mixed therapy groups there is a significant higher percentage of cesarean section comparing with metformin (P value=0.006) (Table 4).

We found that, there is no significant statistical difference between the four groups in neonatal Apgar score at five minutes (Table 5).

Neonatal complications in general, which also considered as causes for admission to NICU, can be single or multiple in the same admitted neonate, and can developed in all infants of diabetic mother, regardless the type of mother's therapy, in our study, we found that, hypoglycemia can occur in neonates of insulin group with highest percentage than others (76.9%), and in lowest with metformin group (44.4%) (p value=0.035), which is significant. Regarding hyperbilirubinemia, also the neonates in insulin group have the highest percent among the others with significant difference (50.0%), (p value=0.008). At the end, the same results were found regarding respiratory distress syndrome, hypocalcaemia, congenital heart disease, neural tube defect, and NICU admission, which showed that there is no significant statistical difference between

the four groups, but RDS, CHD and NICU admission can occur more in insulin group than others (Table 6).

Table 4. Comparison of mode of delivery according to maternal therapy

Parameter		Maternal therapy				P value*
		Insulin N=26	Mixed N=20	Diet N=18	Metformin N=36	
Mode of delivery	CS	19 (73.1%)	17 (85.0%)	16 (88.9%)	18 (50.0%)	0.006
	NVD	7 (26.9%)	3 (15.0%)	2 (11.1%)	18 (50.0%)	
P value**	Insulin vs other groups		0.476	0.270	0.115	
	Mixed vs other groups			1.000	0.011	
	Diet vs metformin				0.007	

* Chi square test, ** Fisher exact test

Table 5. Comparison of Apgar score according to maternal therapy

Parameter		Maternal therapy				P value*
		Insulin N=26	Mixed N=20	Diet N=18	Metformin N=36	
Apgar score	Low	15 (57.7%)	12 (60.0%)	12 (66.7%)	15 (41.7%)	0.284
	Normal	11 (42.3%)	8 (40.0%)	6 (33.3%)	21 (58.3%)	
P value**	Insulin vs other groups		1.000	0.754	0.303	
	Mixed vs other groups			0.745	0.266	
	Diet vs metformin				0.148	

* Chi square test, ** Fisher exact test

Table 6. Comparison of complication according to maternal therapy

Complications	Maternal therapy				P value*
	Insulin N=26	Mixed N=20	Diet N=18	Metformin N=36	
Hypoglycemia	20 (76.9%)	15 (75.0%)	11 (61.1%)	16 (44.4%)	0.035
Respiratory distress syndrome	15 (57.7%)	7 (35.0%)	5 (27.8%)	12 (33.3%)	0.145
Jaundice	13 (50.0%)	6 (30.0%)	4 (22.2%)	4 (11.1%)	0.008
Hypocalcaemia	8 (30.8%)	8 (40.0%)	2 (11.1%)	7 (19.4%)	0.151
Congenital heart disease	7 (26.9%)	3 (15.0%)	0 (0.0%)	2 (5.6%)	0.090
NTD	1 (3.8%)	2 (10.0%)	0 (0.0%)	0 (0.0%)	0.164
NICU admission	23 (88.5%)	17 (85.0%)	13 (72.2%)	25 (69.4%)	0.473

* Chi square and Yates chi square test

Discussion

The occurrence of GDM is increasing in the context of the pandemic in obesity and type 2 diabetes in the modern world, so early diagnosis and treatment can play a significant role in the preservation of the health of mother and her newborn [4]. In this study we compared the different neonatal outcomes according to

the treatment of choice for gestational diabetes mellitus.

Regarding prematurity, there was a statistical significance of preterm labor between metformin and insulin groups (65.4% in the insulin group versus 27.8% in the metformin group), (P value =0.005), which nearly similar to findings in a study at Kashan University of Medical Sciences, Iran, by Mesdaghinia et al. [18], which found a statistical significance of preterm labor between metformin and insulin groups (8 in the insulin group versus 0 in the metformin group (P = 0.007), and the same results in Balani et al. study [19], but Gui et al. study in 2013 [20] mentioned that preterm labor in metformin group is higher than insulin group and this could be due to phenomenon of chance or an unknown effect of metformin on labor cycle. As we know, neonates who are born prematurely have higher rates of cerebral palsy, sensory deficits, learning disabilities and respiratory illnesses compared with those born at term [21]. Regarding neonatal birth weight, we found in this study that the incidence of macrosomia or low birth weight in the metformin group was significantly less than the group receiving insulin (P=0.035) and this agreed with some studies, such as Behrashi et al. [22], their results showed that the incidence of macrosomia in the metformin group was significantly less than the group receiving insulin (P=0.005). Others like Dhulkotia et al. [23] and Zangeneh et al. [24] showed no significant difference between the groups in the prevalence of macrosomia. But Balsells et al. study in 2015 [25], and Cheng et al. [26], found that metformin was associated with a higher birth weight and macrosomia than insulin, suggested that uncontrolled diabetes can lead to fetal macrosomia.

Regarding weight for gestational age, we found also, the percentage of SGA and LGA newborns was higher in the insulin group, compared to metformin group with a significant statistical difference (P=0.045) and this result is in agreement with Simeonova-Krstevska et al. study in Macedonia [8], Goh et al. [27] and Rai et al. [28]. Surprisingly, although mean glycemic values were higher in the insulin group, the percent of SGA newborns was higher. It can be explained by a high incidence of prematurity in the insulin group, as we know, being SGA is as complicated as being LGA, since both are associated with higher morbidity and mortality in the short- and long-term, among the perinatal complications of an LGA newborn, are noteworthy the increased risk of meconium aspiration, clavicle fracture, perinatal hypoxia, hypoglycemia, hyperbilirubinemia, transient tachypnea, brachial plexus injury, shoulder dystocia, and even neonatal death, therefore, preventing the occurrence of both is important [29]. AGA is the treatment goal, and in our study, metformin was associated with high percent (58.3%) rather than insulin therapy (26.9%), and the difference was significant (P=0.045) and this agreed with Goh et al. [27] and Silva et al. study in Brazil in 2017 [30], and like our study, they also, found that there is no significant statistical difference between metformin and insulin in mode of delivery but higher percent of caesarean delivery associated with insulin group than metformin, probably, due to a higher percent of LGA newborns in insulin group, in addition to these results we also found that there is significant statistical difference between diet, mixed groups and metformin group (P=0.007, 0.011) respectively, in cesarean section delivery, this difference may be due to the dissemination of cesarean practice in our country, unlike others.

Fifth minute Apgar score revealed no difference between the four groups statistically, similar results were reported by Rowan et al. [31], and Ijäs et al. [32].

Regarding hypoglycemia, we found that neonates of mothers treated with insulin therapy have higher percentage among other groups (76.9%) and lower in metformin group (44.4%) and this statistically significant (P=0.035), these results were in agreement with the results of Tertti et al. [33], which showed that, the incidence of neonatal hypoglycemia was significantly higher in the insulin group than in the metformin group (P= 0.03), similar results also found by Hellmuth et al. [34], But Gilson and Murphy study in USA [35] showed that the neonatal hypoglycemia was less in the metformin group than insulin, but there was no statistically significant difference

between the two groups. Other studies reported a higher incidence of neonatal hypoglycemia in the metformin group compared with the insulin group and others, but the difference between the groups was not significant, like the results in study by Conway et al. [36] and Ramos et al. in USA [14], they found that this difference may be related to the level of glycemic control in patients in the various studies.

Regarding hyperbilirubinemia, in our study, we found a significant statistical difference among the groups, higher percent was in insulin therapy (50.0%) and lower percent in metformin (11.1%), which considered a significant difference (P=0.008), similar result found by Mesdaghinia et al. [18] and Hyer et al. [37], but regarding respiratory distress syndrome, hypocalcaemia we found that, there is no significant statistical difference between the four groups but can occur more in insulin and mixed groups, which are nearly similar to the results of other studies like Jacobson et al. [38], Behrashi et al. [22] and Tempe et al. [39], findings of these studies showed that there was no statistically significant difference among groups in the prevalence of hypocalcaemia, respiratory distress syndrome and neonatal jaundice. Others such as Mesdaghinia et al. [18], and Hyer et al. [40], found that respiratory distress syndrome and hypocalcaemia among neonates of the insulin group were significantly more rather than the metformin group, this is believed to be related to higher rates of preterm labor in their studies.

Congenital anomalies such as Congenital heart disease and neural tube defect in our study associated more with insulin and mixed therapy group than others, but it revealed no statistical differences between groups and this results agreed with Tertti et al. [33] and Hawthorne [41], but Ramos et al. [14], and Homko et al. [42] reported greater incidence of congenital anomalies in patients treated with metformin than the insulin group, suggested that risk of major congenital abnormalities may be related to maternal glycemic control before and during pregnancy.

In our study, neonatal admission to neonatal intensive care unit up to 1 week after birth due to single or multiple complications has been recorded in the four groups as a high total percentage 78%, and there is no significant statistical value among groups but Insulin group newborns were admitted in more percent than metformin group, Rowan et al. [51] reported similar results, they assumed that higher rate of NICU admission in the insulin group could be due to higher prevalence of preterm labor.

In general, the findings of this study showed that metformin as a treatment of gestational diabetes mellitus has fewer side effects on fetus and newborns with better short- and long-term outcomes than others. This was corroborated in the study carried out by Elliott et al. [43]; in their study, they observed that very low level of metformin could pass through the placenta, also it had the lowest concentration in infants' umbilical cord blood of diabetic mothers under treatment. The reason behind this observation was the strong tendency of the drug to bind to proteins (it is reported as 99.9%) and a very short half-life of 4-6 h [8], in another study carried out by Kraemer et al. [44] to assess the binding effect of metformin to proteins, they found that by removing albumin, blood levels of metformin in umbilical cord still remained undetectable. They concluded that a specific pump actively pumps it into the maternal blood against the direction of fetal blood concentration. This pump, with the two above mentioned mechanisms, has made metformin a suitable drug for the treatment of gestational diabetes with minimal transmission to the fetus.

In conclusion, the pediatrician in the delivery room should expect different neonatal outcomes according to maternal therapy for GDM and their compliance during pregnancy. So, the results of our study, found that metformin can be a good alternative for insulin and others types of maternal therapy in the treatment of GDM. It is associated with better outcomes and less complications for fetuses and neonates.

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Author contribution

Dr. Rashed and Dr Al-Omrani collected the data, Dr. Mohmmed and Dr. Al-Bahadle wrote the first draft of manuscript, all of them share in statistical analysis and discussion.

Conflict of interest

Authors declare that there is no conflict of interest.

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Characteristics and Factors Associated with Hearing loss (Deafness) in Children Attending the Medical City Hospital, Baghdad, Iraq

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Abstract

Background Hearing loss (HL) is one of the commonest and neglected childhood disabilities. According to the World Health Organization (WHO), most of childhood hearing impairment is preventable. Studies showed that 31% of hearing impairment cases are caused by prenatal and postnatal infections, 17% are birth-related causes, 4% are ototoxic medications and about 8% related to other causes such as maternal substance abuse.

Objective To review factors associated with HL in a sample of children attending the Medical City Hospital in the Pediatrics Outpatient Clinic and Auditory Clinic; including congenital, early, or late onset and to investigate risk factors associated with HL.

Methods A cross sectional study, included children <15 years with hearing loss attending the Medical City Hospital in the Pediatrics Outpatient Clinic and Auditory Clinic during the period from the 1st of July 2021 till the 30th of October 2021.

Results One hundred and one children with HL; 65.3% of them were males, 71.3% were preschoolers, 60.4% of them had family history of deafness, Severe deafness was found among 45.5% of children. Management was by Cochlear implantation for 60.4% of them and hearing aid for the rest. Causes of deafness were unknown in 39.6% of children, congenital among in 37.6%, acquired in 15.8% and mixed causes in 6.9%.

Conclusion Hearing impairment is severe deafness in most of the children included in this study. Family history, congenital infections and otitis media were the major causes of hearing impairment. Finally, cochlear implant was the management of choice in the majority of these children.

Keywords Hearing loss, cochlear implant, otitis media

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List of abbreviations: CDC = Center of Disease Control /Atlanta/USA, CMV = Cytomegalovirus, HL = Hearing loss, PTA = Pure tone audiometry, TORCH = Toxoplasma gondii, other agents, such as Treponema pallidum, Varicella zoster virus, Parvovirus B19, and human immunodeficiency virus, Rubella, Cytomegalovirus

Introduction

Childhood hearing loss (HL) is a significant cause of disability worldwide, 466-million persons are living with HL and about 34 million of them are children. It is estimated that by the year 2050, over 900 million persons will suffer from hearing impairment if the current trend continues [(1)].

A child is said to have HL if he/she was not able to hear. HL could be mild, moderate, severe, or profound. It can affect one ear or both ears, and leads in the future to difficulty in hearing, speech or loud sounds. Hard of hearing refers to patients complaining of HL ranging from mild to severe [(2)]. Children who are hard of

hearing are usually able to communicate through spoken language and later on can benefit from hearing aids, cochlear implants, and/or other assistive devices. 'Deaf' children mostly suffer profound hearing impairment, which implies very little or no hearing. They often get benefit from sign language for communication [2,3].

HL is one of the commonest and neglected childhood disabilities because of its gradual onset, often painless and physically hidden nature. The parents usually complain only of behavioral problems, like frequent requests, carelessness, improper responses to instruction, talking too loud and confusion [4]. However, these behavioral complaints are subjective, and are usually ignored by teachers and parents, who consider that these children have no hearing problems [5].

According to the World Health Organization (WHO), most of childhood hearing impairment is preventable; studies showed that 31% of hearing impairment cases caused by prenatal and postnatal infections, 17% are birth-related causes, 4% are ototoxic medications and about 8% related other causes such as maternal substance abuse [6,7].

In children, disabling hearing impairment affects speech and language development and affects children's educational and vocational performance. Furthermore, it causes difficulty in obtaining, performing, and keeping a job, also, stigma, feelings of isolation, and depression, coupled with the poverty, and poor health that create a huge social and economic burden on society worldwide. Without suitable interventions, hearing impairment is a barrier to both education and social integration. These consequences can be reduced by early detection with appropriate audiological and speech interventions [8,9].

Many studies have been done among children and adolescents to assess the prevalence of hearing problems and its associated factors [10], nevertheless, there are great variations in these studies' findings. This demonstrates the demand for a comprehensive investigation of the burden of hearing problems to inform policymakers, program planners, care providers, in addition to stakeholders to serve more efforts on childhood hearing problem especially in developing countries [11,12].

This study aimed to describe risk factors associated with HL in children attending Medical City Hospital in Baghdad, Iraq during the period from 1st of July 2021 to 30th of October 2021, including congenital, early, or late onset hearing loss. Risk factors associated with hearing impairment will be investigated as a secondary aim.

Methods

Study design and sampling

A cross sectional study, included 101 children <15 years old with HL who attended Pediatrics Outpatients Clinic and Auditory Clinic in the Medical City Hospital during the period from 1st of July to 30th of October 2021. The inclusion criteria included those with hearing/deafness problems; for 2 consecutive months, 2 days/week, 5 hours/ day. While exclusion criteria: children admitted to the wards or emergency units for other reasons.

Data collection and instrument

A questionnaire paper was filled by the researcher. It includes the following data:

1- Sociodemographic and clinical features: (Age, gender, parents' job, parents' education, family history, consanguinity, degree of kinship.
2- Diagnosis: First and current chief complaints, time of onset of the illness, current management.
3- Causes of HL (congenital and/or acquired).

Ethical approval

This study was approved by Ministry of Health, Medical City Directorate, and Medical city Hospital Ethics Committee and the requirement for informed consent was waived by the Ethics Commission due to the observational nature of the study. Informed consents were taken from parents.

Statistical analysis

Microsoft Excel 2010 and IBM statistical package for social sciences (SPSS) version 24 were used for data entry, management, and analysis. Descriptive analyses of the variables were expressed as frequencies and percentage for categorical data. While mean of standard deviation was used for quantitative data that is normally distributed, represented by figures and tables. To compare qualitative variables, we utilized the chi-square test, and we used $P<0.05$ to determine statistical significance.

Results

This study included 101 children with hearing loss. Males formed 65.3% of patients while females formed 34.7%. Preschool children (3-5 years) formed 71.3%, while school age children (6-12 years) formed 18.8%, Employed fathers formed 53.5%, 78.2% of them with secondary school education or less. Employed mothers formed 12.9%, and 80.2% of them with secondary school education or less. Parents' consanguinity formed about 86.1% with family history of deafness among 60.4%. Diagnosis of hearing problem was done at 1st year of life among 64.4%. Severe deafness was founded among 45.5% of children while profound deafness was among 43.6%. Deaf children were managed at diagnosis for first time by Cochlear implantation for 60.4% of them, hearing aid for the rest, 39.6%. Reasons behind current children's visit were for programming Cochlear Implants, 40.6%; for cochlear implant maintenance, 7.9%; for pure tone audiometry PTA, 19.8%; and for rehabilitation of hearing aid, 31.7%. Causes of deafness were unknown among 39.6%, congenital among 37.6%, acquired among 15.8%, and mixed causes (congenital and acquired causes) among 6.9%. Other features are shown in table 1.

Table 1. Sociodemographic features of children with hearing loss

Sociodemographic features		N	%
Gender	Male	66	65.3
	Female	35	34.7
Age/ Years	< 3 years	10	9.9
	Preschool age (3-5 years)	72	71.3
	school age (6-12 years)	19	18.8
Father Occupation	Employed	54	53.5
	Not employed	47	46.5
Father Education	> 2ndary school	22	21.8
	=<2ndary school	79	78.2
Mother Occupation	Employed	13	12.9
	Not employed	88	87.1
Mother Education	> 2ndary school	20	19.8
	=<2ndary school	81	80.2
Consanguinity	Positive	87	86.1
	Negative	14	13.9
Family History	Positive	61	60.4
	Negative	40	39.6
Age at diagnosis	< 1 year old	16	15.8
	1st year	65	64.4
	2nd year	11	10.9
	≥3rd year	9	9.0

Figure 1 shows that severe deafness was found among 45.5% of children while profound deafness was among 43.6%. Deaf children were managed at diagnosis for first time by Cochlear implantation for 60.4% of them, hearing aid for the rest, 39.6%.

Reasons behind current children's visit were for programming cochlear implants, 40.6%; for cochlear implant maintenance, 7.9%; for pure tune audiometry (PTA), 19.8%; and for rehabilitation of hearing aid, 31.7%. Causes of deafness were unknown among 39.6%, congenital among 37.6%, acquired among 15.8%, and mixed causes (congenital and acquired causes) among 6.9%.

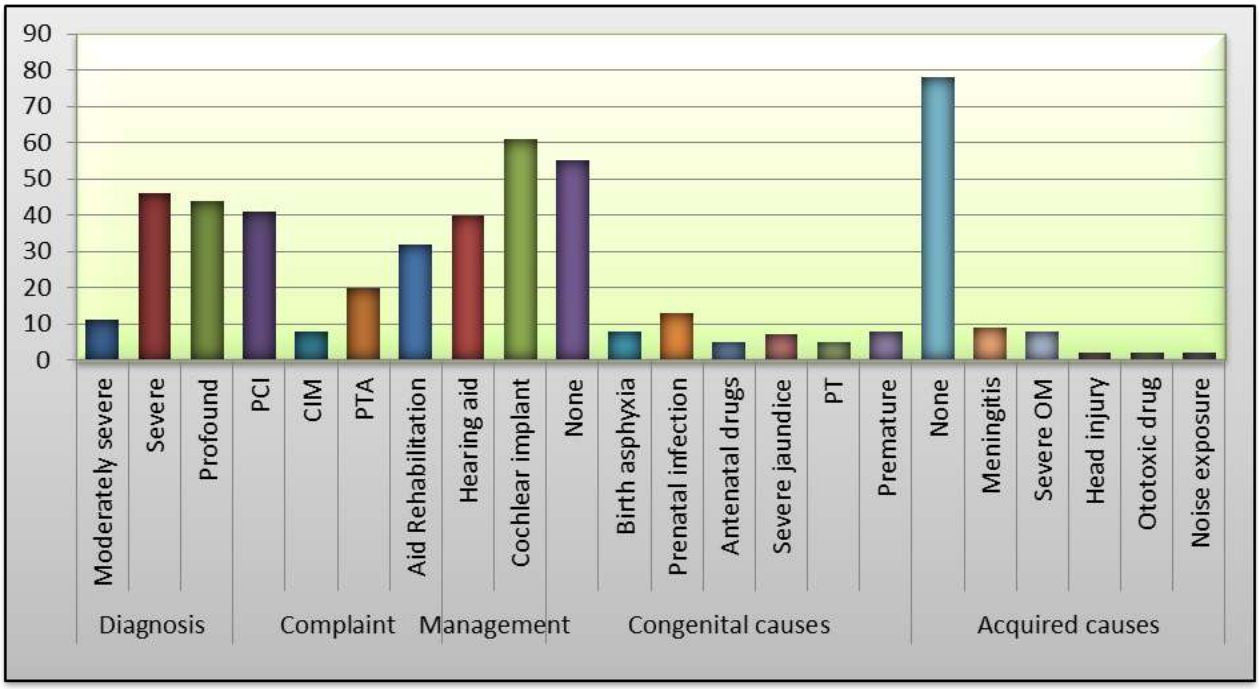

Figure 1. Clinical features of children with hearing loss

Table 2 shows sociodemographic association with different diagnosis. A significant association was founded between severe deafness with preschool age (P<0.05).

Table 3 shows association of causes and management with different diagnosis. A significant association was founded between severe deafness and management with cochlear implant (P<0.05), and between profound deafness with otitis media and noise exposure, P=0.032.

Distribution of studied groups according to different causes was shown in table 4. There was a significant difference between unknown cause of deafness and positive family history, P<0.001.

Table 2. Distribution of patients according to sociodemographic features and different diagnosis

Parameter		Moderately severe deafness		Severe deafness		Profound deafness		P value
		N	%	N	%	N	%	
Gender	Male	7	10.6	27	40.9	32	48.5	0.38
	Female	4	11.4	19	54.3	12	34.3	
Age Group	< 3 years	3	30.0	4	40.0	3	30.0	0.001
	Preschool age (3-5 years)	2	2.8	38	52.8	32	44.4	
	school age (6-12 years)	6	31.6	4	21.1	9	47.4	
Consanguinity	Positive	11	12.6	40	46.0	36	41.4	0.29
	Negative	0	0.0	6	42.9	8	57.1	
Family History	Positive	8	13.1	27	44.3	26	42.6	0.67
	Negative	3	7.5	19	47.5	18	45.0	

Table 3. Distribution of studied group according to different diagnosis

Parameter		Moderately severe deafness		Severe deafness		Profound deafness		P value
		N	%	N	%	N	%	
Management	Hearing aid	11	27.5	13	32.5	16	40.0	<0.001
	Cochlear implant	0	0.0	33	54.1	28	45.9	
Congenital history	None	6	10.9	28	50.9	21	38.2	0.56
	Birth asphyxia	1	12.5	5	62.5	2	25.0	
	Prenatal infection	2	15.4	4	30.8	7	53.8	
	Antenatal use of drugs	0	0.0	3	60.0	2	40.0	
	Severe jaundice during 1st 24 hours of birth	2	28.6	2	28.6	3	42.9	
	Toxemia during pregnancy	0	0.0	2	40.0	3	60.0	
	Premature/low birth weight	0	0.0	2	25.0	6	75.0	
Acquired causes (diseases-disorders)	None	11	14.1	30	38.5	37	47.4	0.032
	Meningitis	0	0.0	8	88.9	1	11.1	
	Severe OM	0	0.0	7	87.5	1	12.5	
	Head injury	0	0.0	1	50.0	1	50.0	
	Ototoxic drug	0	0.0	0	0.0	2	100	
	Noise exposure	0	0.0	0	0.0	2	100	

Table 4. Distribution of studied group according to different causes

Parameter		Unknown		Congenital		Acquired		Congenital and acquired		P value
		N	%	N	%	N	%	N	%	
Gender	Male	25	37.9	27	40.9	9	13.6	5	7.6	0.71
	Female	15	42.9	11	31.4	7	20.0	2	5.7	
Age Group	< 3 years	5	50.0	4	40.0	0	0.0	1	10.0	0.27
	Preschool age (3-5 years)	24	33.3	30	41.7	14	19.4	4	5.6	
	school age (6-12 years)	11	57.9	4	21.1	2	10.5	2	10.5	
Consanguinity	Positive	37	42.5	33	37.9	11	12.6	6	6.9	0.14
	Negative	3	21.4	5	35.7	5	35.7	1	7.1	
Family History	Positive	34	55.7	20	32.8	2	3.3	5	8.2	<0.001
	Negative	6	15.0	18	45.0	14	35.0	2	5.0	

Discussion

Early childhood relationships and interactions with the environment influence social acceptance and a child's ability and skills to form social relationships later in life [13]. These skills are mainly acquired through hearing and talking, the negative consequences of delayed diagnosis for children with HL will affect language, cognitive, and social-emotional skills particularly impaired, also it will delay access to early intervention programs [14].

According to the Center of Disease Control /Atlanta/USA (CDC), about 2 to 3 out of every 1,000 children in the United States are born with a detectable level of hearing impairment in one or both ears [15]. The current study included 101 children with HL, the majority were males, preschoolers, parents' consanguinity formed about 86.1% with family history of deafness among 60.4%, our figure is much higher than the CDC numbers, this is maybe by due to the type of our society with high consanguineous marriages, and the fact that our study was held in a specialized center for cochlear implant.

This is agreed with a retrospective, observational study of newborns in 2010 in Spain that found the percentage of children with a family history and HL was (3.2%), which is higher than expected in the general population [16].

Severe deafness in this study was found among less than half of children while profound deafness was among lesser percentage (43.6%). Cochlear implantation was the management of choice for 60.4% of them, hearing aid for the rest. So, and as a result, visiting for programming cochlear implants and maintenance where the major cause for visit to the hospital. This is reported also by The Non Communicable Disease Control approximately 736,900 cochlear implants have been implanted worldwide by December 2019. In the United States, roughly, 65,000 devices had been implanted in children [17].

This could be understood as management of HL is mainly influenced by the nature, the bilaterally, the severity, the onset and age at diagnosis. Severe to profound bilateral Sensorineural hearing loss can be managed by cochlear implantation weather unilateral or bilateral if picked up at early age of the child, while mild to moderate bilateral hearing loss are easier to manage with conventional hearing aids [18].

In our study, causes of deafness were unknown in the largest percentage, one third of them had mixed cases congenital and acquired, 6.9% of them had prenatal infection (TORCH) (*Toxoplasma gondii*, other agents, such as *Treponema pallidum*, *Varicella zoster* virus (VZV), Parvovirus B19, and human immunodeficiency virus (HIV), Rubella,

Cytomegalovirus (CMV)), *Herpes simplex* virus (HSV)) and birth asphyxia were common congenital causes of deafness, while meningitis and severe otitis media were most common acquired causes among same age group. This high percentage of unknown causes are probably due to the cost of the genetic analyses required, lack of awareness about many prenatal infections.

A recent survey by CDC reported that genes are attributed for hearing impairment among 50% to 60% of children presented with HL. About 20% of infants with genetic HL come with accompanied "syndrome" (Down syndrome or Usher syndrome) [15]. on the other hand, maternal infections during pregnancy, environmental causes, and some events after birth are responsible for hearing problems among about 30% of children with HL. It is also found that congenital CMV infection during pregnancy is a common preventable risk factor among children. Unfortunately, 14% of those exposed to CMV during pregnancy develop sensorineural HL. A health styles survey by CDC in 2005 reported that only 14% of female participants had heard of CMV and had knowledge regarding other TORCH infections [19].

Concerning acquired causes, another study included reports of registered hearing aids devices of the United States Food and Drug Administration in December 2019 demonstrated that five out of six children experience ear infection (otitis media) by the time they are 3 years old. In many developing regions, middle ear disease abounds and often is of the dangerous suppurative type. Certain populations had intermediate, with acute suppurative and chronic otitis media being almost endemic, yet rarely cholesteatomatous [20].

The present study found that preschool age (3-5 years) shows a significant association with severe deafness and cochlear implant as management, another significant association was founded between severe deafness with preschool age and management with cochlear implant, and between profound deafness with otitis media and noise exposure.

This is disagreed with the results of a study involving a population-based data that collected children with HL from 2003 to 2013, in which the multivariate analysis showed no statistically significant relationship between risk factors examined by the researchers (TORCH, family history, syndromes, and postnatal infections) and the probability of the presence of progressive HL. However, the presence of congenital anomalies was inversely associated with progressive hearing impairment [21]. Providing that this study was held in a specialized center of hearing problems and cochlear implants, comparison to population studies may not provide a full picture of the variability among those studies worldwide.

Without population-based screening, it is difficult to diagnose whether HL occurred congenitally, in neonatal period, or during early childhood [23]. In our country, accurate information and registration on the progression of HL is also difficult to obtain due to limited data on hearing loss onset. In addition to newborn screening for HL, there is a vital need to monitor under five children who are at risk of developing HL beyond the neonatal period. Also, using that data in many countries in early hearing detection and Intervention programs, campaigns and care had provided essential services to these children in need and their families during COVID-19 [22,24].

In conclusion, hearing impairment in children in this study is of the sever deafness in most of them and cochlear implant was the management of choice in the majority. Family history, congenital infections and otitis media were the major causes of hearing impairment.

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Author contribution

Dr. Qasim: study design, data collection. Dr. AbdulKareem: writing, data-analysis, Dr. Issa: review and editing.

Conflict of interest
None.

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Review of HPLC Methods for Determination of Azithromycin in Different Samples

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Abstract

Azithromycin is a semi-synthetic macrolide antibiotic of the azalide groups. It inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit of the bacterial 70S ribosome. It inhibits peptidyl transferase activity and interferes with amino acid translocation during the process of translation. Its effect may be bacteriostatic or bactericidal depending on the organism and the drug concentration. Azithromycin is one of the famous and important antibiotics agents and the determination methods of azithromycin in this article were tabulated with lots of chemical and instrumental methods that used in different parameters. Different high performance chromatographic methods have been reviewed in this paper.

Keywords Azithromycin, HPLC

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List of abbreviations: FIA = Flow injection analysis, FTIR = Fourier transform infra-red, GC = Gas chromatography, HPLC = High performance liquid chromatography, NMR = Nuclear magnetic resonance, UV = Ultraviolet

Introduction

High performance liquid chromatography (HPLC) is a procedure that is used for separation a mixture into its fractions or components. It is a separation method and the separated components identified by using any analytical method such as ultraviolet (UV)-visible, Fourier transform infra-red (FTIR), mass spectroscopy, nuclear magnetic resonance (NMR) etc. For quantitative analysis, the measurement of the area under the curve or peak height in the chromatogram is done. These bands or peaks are formed due to the separation of the compounds using different lengths on the chromatographic columns in HPLC and gas chromatography (GC) and on paper or thin layers in paper chromatography. The principle of HPLC is include that the samples are injected to flow by a mobile liquid phase via the particles of stable stationary phase. The compounds are separated into individual components based on their affinity towards the two phases during their flowing [(1)].

There are two types of separation in HPLC; isocratic elution, in which composition ratio of the mobile phase is keep same through the analysis and gradient elution, in which composition is subjected to change during the separation of sample [(2,3)]. The instrumentation of HPLC consists of mobile reservoir phase, pump, column, detector and recorder as shown in figure (1) [(4-7)].

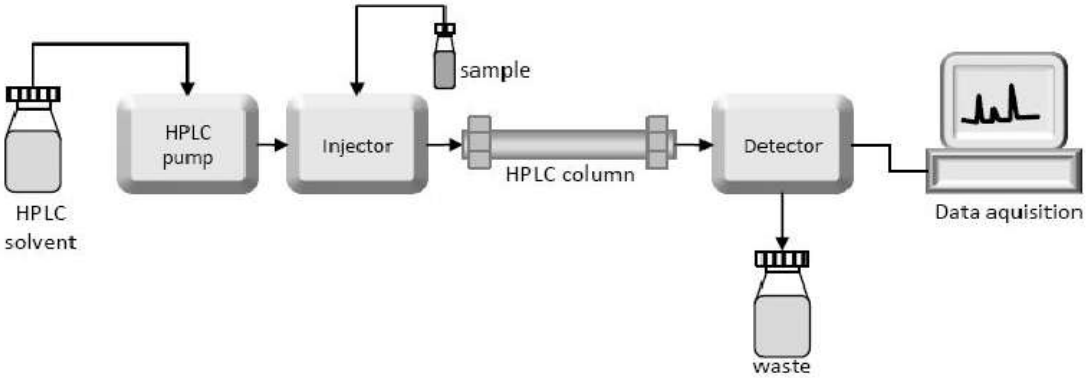

Figure 1. The instrumentation of HPLC [7].

The applications of HPLC are for qualitative and quantitative analysis, direct comparison method, calibration curve method, internal standard method, checking the purity of a compound, presence of impurities and determination of mixture of drugs [8,9].

Azithromycin is a 15-membered-ring macrolide that differs from erythromycin by the presence of a methyl-substituted nitrogen in the macrolide ring. Azithromycin (9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin) as shown in figure (2) is derived structurally from erythromycin A by change the 9a carbonyl in the aglycone ring for a methyl-substituted nitrogen, and expansion of the ring to 15 members. This structural difference blocks the internal reaction to form the hemiketal, leaving acid hydrolysis of the ether bond to the neutral cladinose sugar as the main decomposition pathway. At 37°C and pH 2 with ionic strength = 0.02, azithromycin is hydrolyzed with 10% decay in 20.1 min, whereas the equivalent value for erythromycin is only 3.7 sec. The energy of activation for hydrolysis of the ether bond linking cladinose to azithromycin is about 25.3 kcal/mol; while, the internal dehydration reaction of erythromycin has an activation energy about 15.6 kcal/mol [10-15].

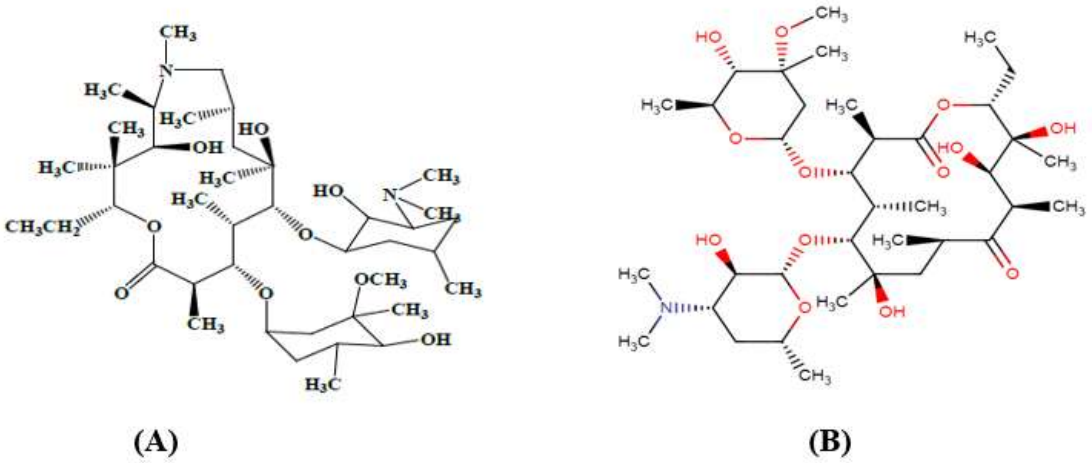

Figure 2. Chemical structure of Azithromycin(A) and Erythromycin(B) [12].

It is used against a variety of bacteria such as *Haemophilus influenzae*, *Streptococcus*, *pneumoniae*, *Mycoplasma pneumoniae*, *Staphylococcus aureus*, *Mycobacterium avium* [16]. It has the ability to prevents these bacteria from growing by interfering proteins synthesis. According to differences in protein synthesis between bacteria and humans, these antibiotics do not interfere with production of proteins in humans [17]. It is a unique antibiotic that stays in the body for quite a while (has a longer half-life) allowing for once-a-day dosing and for shorter treatment courses for most infections [18]. Azithromycin is absorbed quickly after oral administration with a bioavailability about 36%. It has a various effect with food. It is also as a poorly water-soluble drug [19,20]. In this review article, we are tabulating the most recent HPLC methods that were used for determination of azithromycin in different formulations.

Methods and methodology of HPLC

The recent HPLC methods that were used for determination of azithromycin in pure, dosage forms and biological samples were based on efficiency on using the mobile phase and type of columns (stationary phase). These methods are summarized in table (1) including type of mobile phase, type of stationary phase, flow rate, retention time, linearity and detection limit [21].

Table 1. HPLC methods used for determination of Azithromycin

Mobile Phase	Stationary Phase	Flow rate	Retention Time	Linearity	Detection Limit	Ref.
Consist a mixture of 0.0335 M Phosphate Buffer (pH 7.5) and methanol in the proportion 20:80	C-8, 250 mm X 4.6 mm, 5 µm	1.2 ml/min	8.35 min	49.32-148.69 ppm	52.24 ppm	22
Consisting of Acetonitrile: Methanol: Phosphate buffer (40:40:20 v/v)	C18 (150×4.6 mm, 5 µm) column was used	1.0 ml/min	2.95 min	10-50 ppm	2.12 ppm	23
Methanol-phosphate buffer, pH 7.5 (80:20, v/v)	C18, 5 mm, 25 cm length, 4.6 mm	2.0 ml/min	--	0.3-2 mg/ml	0.0005 mg/ml	24
Ammonium acetate (0.05 M, pH 8.0) and acetonitrile (60:40, v/v)	250 x 4.6 mm, with 5 mm particle size and pore diameter 100 A, Boston pHlex ODS	0.8 ml/min	16 min	50.9-509.3 ppm	6.75 ppm	25
Buffer, acetonitrile and methanol (60:20:20) adjusted to pH 8.1 with phosphoric acid	C18 column	1.0 ml/min	5.23 min	--	--	26
Acetonitrile-0.1 M KH_2PO_4 (pH 6.5–0.1) M tetrabutyl ammonium hydroxide pH 6.5-water (25:15:1:59)	XTerra® (250 mm x 4.6 mm i.d., 5 µm particle size)	1.0 ml/min	8 min	50-150%	0.02%	27
It is consisting of acetonitrile, methanol, phosphate buffer, 0.05 M, pH 6.0 (20:20:60)	It is Eclipse XDB-CNTM 5 µm, 150 x 4.6 mm (Agilent Technologies, Palo Alto, CA) protected by a guard column Xterra RP18, 3.9x 20 mm	1.0 ml/min	16.6 min	10-400 ng/ml	--	28

Consist of methanol, acetonitrile and phosphate buffer pH 8 (60:30:10)	It is C18 (250 mm × 4.6 mm i.d.)	1.0 ml/min	4.8 min	1-50 ppm	14.40 ng/ml	29
It is 35 mM ammonium acetate buffer (mobile phase-A) and acetonitrile and methanol in ratio of 90:10 (as mobile phase-B)	Luna C18 (3 µ, 2x150 mm) column	0.25 ml/min	7.8 min	0.5-50 ng/ml	--	30
Consist of methanol/buffer mobile phase at the ratio of 90:10	C18 column, 5 µm, 250 ×4.6 mm	1.5 ml/min	7.23 min	1-80 ppm	0.3 ppm	31
Contains Ammonium acetate solution (30 $mmolL^{-1}$, pH= 6.8) and acetonitrile (18:82, v/v)	It is Hypersil GOLD C-18 analytical column packed with deactivated silica (250 mm x 4.6 mm ID x 5 µm)	0.7 ml/min	7.95 min	5-200 ppm	0.476 ppm	32
Contains acetonitrile and phosphate buffer (pH 11 ± 0.05) of 60:40 (v/v)	It is Shodex ODP-50 column (250×4.6 mm i.d., 5 µm particles)	1.0 ml/min	7.34 min	--	--	33
Consisting of acetonitrile –2-methyl-2-propanol-hydrogenphosphate buffer, pH 6.5, with 1.5% triethylamine (33:7: up to 100, v/v/v)	End-capped ODB RP18 column	1.0 ml/min	12.83 min	0.25-15 ppm	0.37 ppm	34
it is acetonitrile –2-methyl-2-propanol–hydrogenphosphate buffer, pH 6.2, with 1.8% triethylamine (32:8: up to 100, v/v/v)	Using C18 ODB column (250×4.6 nm i.d.)	1.1 ml/min	12.35 min	0.004-4.8 mg/ml	0.02%	35
A mixture of Methanol and 0.0335M Phosphate Buffer (pH 7.5) (80:20 v/v)	It is ODS C18, 250 x 4.6 mm, 5 µm, L1 packing, column	1.2 ml/min	3.83 min	0.1-12 ppm	1.6 ppm	36
Ammonia buffer with pH = 6.7 (A) and acetonitrile (B)	Column Ascentis® with the length of 150 mm, inner diameter of 2.1 mm and particle size of 2.7 µm	0.6 ml/min	2.8 min	2.5-400 ng/ml	0.7 ng/ml	37

In general, several analytical techniques for the analysis of Azithromycin have been presented. However, further efforts to use widely modern chromatographic techniques HPLC coupled with tandem mass spectrometry for the quantitative analysis of Azithromycin. The main goals to be addressed in the future include improved selectivity, sensitivity, analytical simplicity, and efficiency of the HPLC method.

In conclusion, in this review article we conclude that Azithromycin can be determined in different samples using accurate analytical methods such as HPLC also these methods can be attached to different instruments such as flow injection analysis (FIA) and

spectrophotometry. We also found from the above summarized methods in table (1) that each method has it is advantaged like type of sample, cost of mobile and stationary phases beside the accurate of the obtained results. All the researchers can used any of these methods to qualitative and quantitative of azithromycin in it is sample.

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The Impact of Very Late Antigen 4 Polymorphism on Drug Responsiveness in Patients with Multiple Sclerosis Initiation

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Abstract

Background Very late antigen 4 (VLA4) integrin facilitates the immune cells migration to central nervous system (CNS) through blood brain barrier (BBB), so the polymorphism in this gene may be considered as genetic risk factor for multiple sclerosis (MS) occurrence. It may interact with the responsiveness level of Natalizumab.

Objective To show if VLA4 single nucleotide gene polymorphism (SNP) (C-269-A) considered as genetic predisposition factor for MS and if have a role in Natalizumab (Tysabri) drug non-responsiveness.

Methods Sixty-six (66) person with MS and 60 healthy persons involved in this study, their ages were range from 14 to 67 years. They attended to seek treatment in the MS outpatient's clinic, at Baghdad Teaching Hospital, Medical City Complex from December 2018 to March 2020. Patient were divided into two group; resistant group (34) and response group (32). The VLA-4 SNP polymorphism investigated by sequence specific primer polymerase chain reaction (SSP-PCR) technique.

Results The VLA4 gene SSP-PCR genotyping revealed no significant differences between patients and control group also between responder patients and non-responder to Natalizumab.

Conclusion VLA-4 polymorphisms at the level of SNP at positions 269 (C/A) have no role in MS susceptibility or Natalizumab responsiveness.

Keywords MS, Natalizumab, VLA-4

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List of abbreviations: SSP-PCR = sequence specific primer polymerase chain reaction, VLA = Very late antigen, VCAM-1 = Vascular cell adhesion molecule

Introduction

Multiple sclerosis (MS) is the most common neurological immune-mediated disorder of the central nervous system (CNS) that affects patients in the most active and productive time of their lives by causing physical disability and mental retardation [(1)]. The disease is characterized by inter-individual differences in its course and response to immunomodulatory therapy [(2,3)]. It believed that MS is initiated by immune dysregulation triggered by genetic and environmental factors [(4)].

The very late antigen 4 (VLA-4) (alpha 4: beta 1; CD49d / CD29) - approved symbol (ITGA4) integrin is a gene with protein product involved in both cellular adhesion to extracellular matrix and cell-cell interactions [(5)].

Integrin α4β1 (VLA 4) is an integrin dimer. It is consisting of CD49d (alpha 4) and CD29 (beta 1), it is expressed on the cell surfaces of

progenitor cells, stem cells, T cells, B cells, natural killer cells, monocytes, eosinophils and neutrophils. It functions to consolidate an inflammatory response by the immune system through assisting in the movement of leukocytes to tissue that requires inflammation, and it is the main player in cell adhesion [6,7]. After a chemotactic agent or other stimuli activate the leukocytes, the VLA-4 will adhere to its appropriate ligand. The primary ligands of VLA-4 include vascular cell adhesion molecule 1 (VCAM-1) and fibronectin [8].

In MS, the VLA-4 integrin is essential for T cell passing to the brain. It allows the cells to penetrate the blood brain barrier (BBB) that normally limits immune cell access. In many studies, the researchers found the severity of MS is positively correlated with the expression of alpha four. One approach to prevent an autoimmune reaction has been to block the action of VLA-4 so that self-reactive T-cells are unable to enter the brain and thus unable to attack myelin protein [9].

The polymorphic α4-subunit of VLA-4 gene represents a good target for association with MS. Previous study investigated the association between VLA-4 gene polymorphisms and MS on 275 patients and 255 controls, focused on two genetic polymorphisms of the α4-subunit; the first, a single point mutation at position 3061 producing an arginine (CGG) to glutamine (CAG) trans version, the second, a C to A transversion at position 269 in the promoter region of exon [10].

Natalizumab (NTZ) is a humanized monoclonal IgG4 antibody blocking the a4-integrin subunit, its acts as a α4 integrin antagonist to prohibit leukocyte trafficking into the central nervous system [11,12]. It is approved by United States Food and Drug Administration (FDA) for the treatment of relapsing–remitting multiple sclerosis (RRMS). Recently, a study established to identify pharmacogenetic factor(s) associated with MS patients' response to NTZ, this study found that the variant (rs2304166) in GP6 gene is associated with poor response to NTZ in homozygous CC genotype MS patients [13].

So, we designed this study to identify the genetic impact of VLA-4 gene on MS initiation and NTZ responsiveness.

Methods

Sixty-six patients (66) with MS were involved in this case control study. Their ages were range from 14 to 67 years. They were attended for seeking treatment in the MS outpatient's clinic, at Baghdad Teaching Hospital, Medical City Complex in the period extended from December 2018 to March 2020.

They diagnosed according to McDonald criteria [14] by a neurologist and the diagnosis confirmed by magnetic resonance imaging and some cases by oligoclonal band test in the cerebrospinal fluid. Patients were subjected to questionnaire about name, age, sex, smoking, family history, medication, number of relapses in the last year, type of medication, and first clinical signs during diagnosis.

According to Rio criteria [15], the patients were divided into two groups, group I (32) responder to NTZ (Tysabri) and group II (34) non-responder to NTZ (Tysabri). The Institutional Board Review (IRB) committee of College of Medicine, Al-Nahrain University approved this study, and all samples were obtained with permission of Ministry of Health declaration.

After explaining the objective of the current study and agreed to accession of the study, sixty volunteers were involved as controls, their sex and ages were matched with patients' group were included in this work as control. All of them received no treatment with no complaint of other chronic or systemic diseases; not suffering from any neurological signs in the last 2 years their age range was (16-68) years.

Inclusion criteria

Multiple sclerosis patients on NTZ for more than 1 year.

Exclusion criteria

We excluded the patients whom not stick to treatment and have a period of treatment discontinuous.

The detection of ITGA4 single nucleotide gene polymorphism (SNP) rs113276800 (−269C/A) in the present study was done by the amplification-refractory mutation system (ARMS). Two ml of venous blood were drawn from patients and controls in EDTA tube for DNA extraction, which used in SSP-PCR for 66 patients and 60 control, the DNA kept in Eppendorf tube -20℃ till used.

Kits

1. DNA extraction Kit (Geneaid, Taiwan)
2. PCR Kit (Bioneer, Korea)

Procedure

Molecular detection of ITG4 SNP- rs113276800 (−269C/A) in blood sample was done by polymerase chain reaction ARMS. The master mix which used is ready master mix (Accupower PCR premix/ Korea). One microliters of each primer (foreword and reverse as in table 1) and three microliter of template DNA were added to the master mix tube. The final volume was adjusted to 20 ul with free nuclease distal water. The mixture was then vortexed for 10 seconds and put in thermocycler (Bioneer, Korea), which was previously programmed with the following (Table 2).

Table 1. VLA4 primer sequence

Polymorphism	Primer	Sequence	Product length	Method
C 269-A EX 1	269 C	5′-ACGCTCCGCCCGCGGTGGGC-3′	251 bp	PCR-SSP (ARMS)
	269 A	5′-ACGCTCCGCCCGCGGTGGGA-3′		
	269 R	5′-CAGCAACAGCATCACCGTCT-3′		

Table 2. VLA-4 gene amplification PCR program

Temperature	Time	Cycle
95°C	5 minutes	1X
94°C	45 seconds	
61°C	30 seconds	30 X
72°C	30 seconds	
72°C	7 minutes	1 X

Statistical analysis

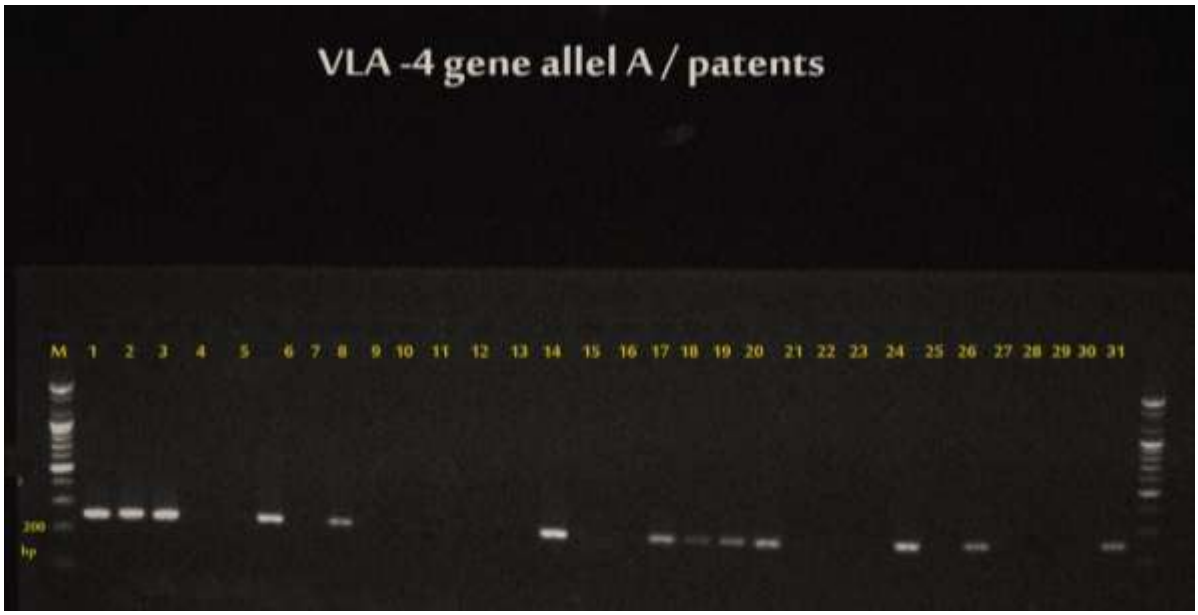
The statistical package for the social sciences V26 (SPSS Inc., Chicago, USA) was used. By comparing the observed and expected frequencies (Chi-square test), the polymorphisms were tested for deviation from Hardy Weinberg Equilibrium (HWE). The association between genotype and risk of MS and drug responsiveness was valued by calculation of odds ratio (OR) with 95% confidence interval (95% CI). Statistical significance was set at a p value <0.05.

Results

There were no significant differences between patients and control in the frequency of different age group.

The ARMS was used to amplify the VAL4 gene using specific set of primers. The PCR products are shown in figure 1 (A & B).

A



B

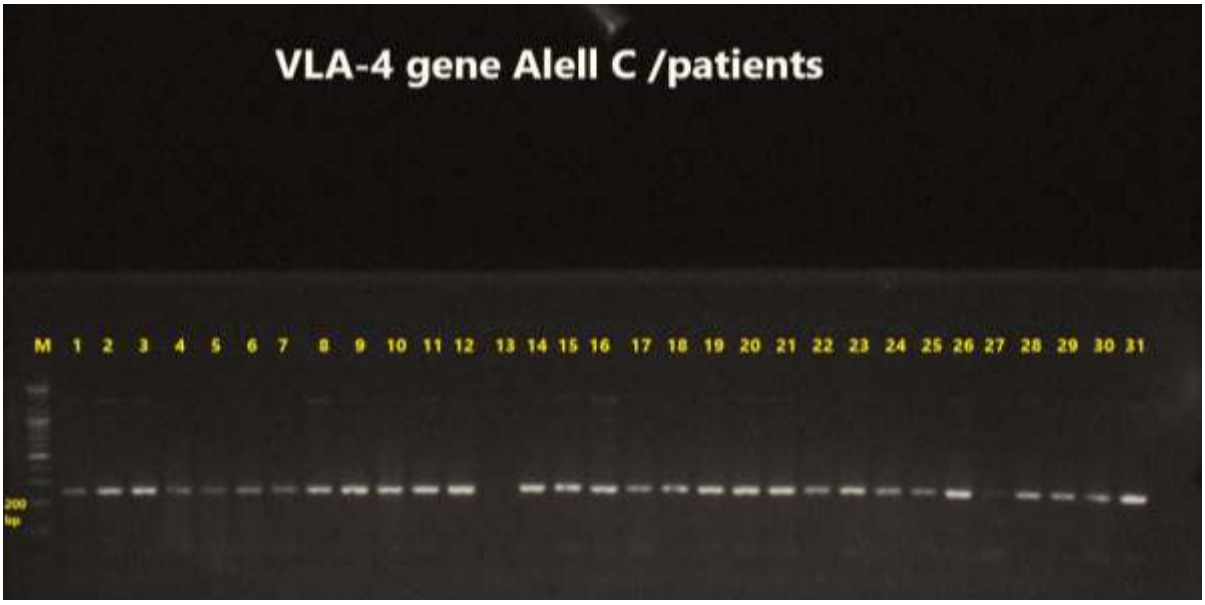


Figure 1. PCR products of VLA-4 Gen; A: A allele in patients, B: C allele in patients

The genotype and allele frequency of VAL4 SNP in patients and controls are shown in table 3. The frequency of different genotypes and alleles of this SNP was almost similar between patients and controls with no significant differences.

Likewise, the distribution of genotypes and allele of VAL4 SNP in responsive and non-responsive patients was almost identical with no significant differences (Table 4).

Table 3. The genotype and allele frequency of VAL4 SNP in patients and controls

VAL4		Controls (50)	Patients (66)	*P*-value	OR (95% CI)
Genotypes	CC	26 (52.0%)	34 (51.52%)	0.587	1.0 Reference
	CA	23 (46.0%)	28 (42.42%)	0.330	0.33 (0.03-3.1)
	AA	1 (2.0%)	4 (6.06%)	0.302	0.3 (0.03-2.92)
	HWE	0.109	0.573		
Alleles	C	75 (75.0%)	٩٦ (72.73%)	0.697	1.0 Reference
	A	٢٥(٢٥.0%)	٣٦ (27.27%)		1.13 (0.62-2.04)

Table 4. The frequency of different genotypes and allele of VAL4 polymorphism in responsive and non-responsive patients

VAL4		Responsive (32)	Non-responsive (34)	*P*-value	OR (95% CI)
Genotypes	CC	16 (50.0%)	18 (52.94%)	0.972	1.0 Reference
	CA	14 (43.75%)	14 (41.18%)	0.911	0.89 (0.11-7.06)
	AA	2 (6.25%)	2 (5.88%)	1.000	1.0 (0.12-8.13)
	HWE	0.346	0.736		
Alleles	C	46 (71.88%)	50 (73.53%)	0.831	1.0 Reference
	A	18 (28.12%)	18 (26.47%)		0.92 (0.43-1.98)

Discussion

As MS initiation needs to cross of immune cells into the CNS, the VLA-4 gene may be considered as a probable candidate genetic risk factor for susceptibility to MS. Therefore, the current work studied the association between SNP at positions 269 (C/A) in the VLA4 gene and the risk of MS. Only a few studies have analyzed the genetic predisposition of VLA-4 (α4β1 integrin) to chronic inflammatory diseases of CNS, including MS.

Ďurmanová et al. (2018) studied the ITGA4 gene polymorphism encoding the VLA-4 α4 subunit with increased risk of Alzheimer's disease, they observed no statistically significant differences in concern ITGA4 −269C/A gene polymorphism (rs113276800) between patients and control [16]. Taher et al (2018) have investigated the rs1143676 (+3061A/G) of VLA-4 gene polymorphism and its association with MS risk in Iranian population, their result showed significant differences in genotype and allele frequencies between the MS patients and healthy subject [17]. Correia et al. (2009) found an association between rs155100 SNP located in the intron 9 of the integrin α4 gene and autism [18].

The current study showed no significant differences between MS patients and healthy control group in concern to C/A transversion at position 269 in the promoter region of exon 1 , this result also obtained by Andreoli et al. (2007), Ďurmanová et al. (2015) [10,19].

On the other hand, the present study revealed the homozygous AA genotype was detected in 2% and 6.06% in control and patients respectively. This outcome disagrees with Hilger-Eversheim et al. (2000), who suggested that no homozygous 269 AA genotype could be observed as the 269 (C/A) polymorphism is

located in the α4 promoter region near the AP-2 binding sites, the AA variant may be responsible for the negative gene expression causing the functional impairment of the α4 subunit [(20)].

In the present study, the 269 (C/A) polymorphism genotyping showed no significant differences between the responder and non-responder patient. This polymorphism never studied as a cause of NTZ (Tysabri) unresponsiveness.

Until 2019, there was only two studies analyzed the pharmacogenetic reasons of NTZ (Tysabri) unresponsiveness [(21,22)]. Recently, a study has established to identify pharmacogenetic factor(s) associated with MS patients' response to NTZ (Tysabri), which found that the variant (rs2304166) in GP6 gene is associated with poor response to NTZ (Tysabri) in homozygous CC genotype MS patients. Al-Mojel et al. (2019) investigated the possible inference of genes encoding detoxification enzyme GSTP1 and NQO1 polymorphisms on NTZ (Tysabri) response in MS, this study concluded a significantly increased frequency of double NQO1 an2d GSTP1 mutant polymorphisms in non-responders compared to the responders [(23)].

In conclusion, VLA-4 polymorphisms at the level of SNP at positions 269 (C/A) have no role in MS susceptibility or NTZ responsiveness.

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Author contribution

Khaliel and Dr. Abbas designed the study and did the laboratory works. Dr. Abdulamir designed the primers. Dr. Hatem determined the responsiveness to Natalizumab criteria and detected the responsiveness group and the non-responsiveness group.

Conflict of interest

Authors declares there is no conflict of interest.

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Study the Effects of (8-oxo-Guanine DNA Glycosylase 1 rs1052133) Polymorphism on Gene Expression as a Predisposing Factor for DNA Mutation in Midland Refineries Company-Dura Refinery Workers

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Abstract

Background Refineries are exposed to different types of air pollutants, among them, polycyclic aromatic hydrocarbons (PAHs), they are a group of over hundred different kinds of hazardous organic chemicals (pollutants), which are formed primarily during the incomplete burning of oil, gas and coal. PAHs are metabolized in different organs of human body especially in the liver leading to generation of mutagenic metabolites, of them, the reactive oxygen species, which damage DNA by binding with guanine base leading to formation of 8-hydroxyguanine. The 8-oxo-guanine DNA glycosylase (hOGG1 rs1052133) gene, is a regulatory gene binds with 8-oxo-DG in the DNA to initiate base excision repair (BER) pathway.

Objective To investigate the role of (hOGG1 rs1052133) gene polymorphism in single nucleotide polymorphism repair.

Methods One hundred sixty-eight (168) subjects were participated in this study. They were they divided into three groups (country side, office workers and field workers). PAHs were analyzed in all participant by Gas Chromatography Coupled with a Mass Spectrophotometry GC/MS. SNP for hOGG1 and gene expression were detecting by real time polymerase chain reaction and quantitative polymerase chain reaction for each subject respectively.

Results PAH were detected in blood serum of refineries workers and not detected in country side participants. The level of hOGG1 gene expression was higher in GG genotype than GC and CC genotype.

Conclusion Refineries worker are at high risk of developing malignant transformation and serious disease due to high level of PAH compared to other population. hOGG1 plays an important role in DNA repair following DNA mutation.

Keywords Poly cyclic aromatic hydrocarbons, HOGGI, gene expression

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List of abbreviations: BER = Base excision repair, ELISA = Enzyme-linked immunosorbent assay, GC/MS = Gas chromatography coupled with mass spectrometry, hOGG1 = 8-oxo-guanine DNA glycosylase, oh8Gua = 8-hydroxyguanine, PAHs = Polycyclic aromatic hydrocarbon, ROS = Reactive oxygen species, SNP= single nucleotide polymorphism

Introduction

Air pollution is the major environmental hazard all over the world, as well as urban areas, associated with increased

morbidity and mortality rates. Refineries worker are exposed to different types of air pollutants, among them polycyclic aromatic hydrocarbons (PAHs) are the common. PAHs are a class of organic chemical substances that contain 2 to 7 fused aromatic rings. Several PAHs are very well known as mutagens, carcinogens and teratogens, and thus pose a serious threat to the global health environment. The physicochemical properties of PAHs make them highly mobile in the environment, allowing them to distribute across air, soil, and water [1]. PAHs are carcinogenic environmental pollutants resulting from incomplete combustion that are commonly found in tobacco smoke, ambient and indoor air, charbroiled foods and oil refinery. After exposure, these compounds are metabolized in human body (liver, kidney and lungs) [2,3]. Reactive metabolites (e.g., epoxides and dihydrodiols) of some PAHs have become one of the major health concerns because of their potential to bind to cellular proteins and DNA with toxic effects, even more toxic than PAHs itself [4]. The resulting biochemical disruption and cell damage can lead to mutations, developmental malformations, tumors, and cancer. Evidence indicates that mixtures of PAHs may be more carcinogenic to humans than individual PAHs. The evidence comes primarily from occupational studies of workers exposed to mixtures of PAHs. According to the U.S. Environmental Protection Agency, seven PAH compounds have been classified as probable human carcinogens: benz(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k) fluoranthene, chrysene, dibenz(ah)anthracene, and indeno(1,2,3-cd) pyrene [1].

In vitro and in vivo, 8-hydroxyguanine (oh8Gua), (a main form of oxidative DNA damaged caused by reactive free radicals that generate from PAHs metabolism, is extremely mutagenic) [5]. The occurrence of oh8Gua in DNA results in a G:C to T: A transversion because oh8Gua controls the incorporation of cytosine and adenine nucleotides in the opposite direction of the lesion [6]. The base excision repair (BER) of the oh8Gua lesion in human DNA is 8-oxo-guanine DNA glycosylase (hOGG1) that initiate the first step of the repair pathway. hOGG1 has been extensively studied since its initial cloning and characterization in 1997 to better understand its catalytic mechanism and the roles of specific amino acid residues in this process. So far, it has been discovered that a few key residues are required to varying degrees for the enzyme's functionality in both substrate recognition and the subsequent catalytic process [7,8].

The hOGG1 rs1052133 gene, located on human chromosome 3p26.2 [9], is a regulatory gene binds with high affinity to 8-oxo-DG especially at guanine-rich promoter regions because the oxidation potential of guanine is the lowest among the four DNA bases in the DNA double-stranded to initiate BER pathway. As well as, hOGG1 has variable signal transduction functions, interacts with 8-oxo-DG in gene regulatory regions, and facilitates gene expression [8,10]. Because of the characteristics of single nucleotide polymorphism (SNP), the interaction of gene mutation and environment may result in DNA repair defects and, as a result, an increase in the happening of certain tumors. hOGG1 genetic polymorphism has been detected in various populations [11].

This study aimed to investigate the role of hOGG1 rs1052133 gene polymorphism in SNP repair.

Methods

Subjects and study groups

One hundred sixty-eight (168) male subjects of Midland Refineries Company - Daura Refinery workers with age between (25-60 years) with healthy (mentally and physically) were participated in this study, a case control study, anywhere carcinoma and subjects with autoimmune disease were excluded, the samples we collected from February to October 2020: these study populations are subdivided into three groups.

Group one: control (lives in country sides (rural)) away from pollution (n=56).

Group two: office workers (who work far of the refinery field (around field)) (n=56).
Group three: field workers (close to the pollution) (n=56).

Specimens' collection

About 10 ml of venous blood was drown from each participant. It was divided to:

A. Seven ml for serum sample (for PAHs determination).
B. Three ml for whole blood sample for:
    - Real-time polymerase chain reaction (RT-PCR) for SNP of hOGG1 gene.
    - RNA extraction (100 µl in 300 µl TRI Reagent®) for hOGG1 expression.

Methods

Determination of PAHs Concentration in Serum by Gas Chromatography Coupled with a Mass Spectrophotometry (GC/MS)

Liquid-liquid extraction technique used for PAHs separation in serum, a volume of (40 ml) n-hexane and (10 ml) dichloromethane (DCM) was used for extraction. The extractant (10 ml) and plasma samples (2 ml) were added in the vials.

The vials were capped and vortexed for 20 sec at 300 rpm. The organic layer was sucked out using pipette attached with pipette filler into clean and thermally treated amber bottles. The extracts were cleaned up in a column (1 cm x 15 cm, internal diameter and length) with slurry of silica gel as stationary phase, preconditioned by distilled water and hexane/DCM before the samples were eluted, collected and concentrated in a stream of nitrogen gas [12].

After extraction, the PAH was analyzed and detecting by GC/MS. A Shimadzu QP 2010 plus GC/MS equipped with an auto-injector AOC-20i have been used for this purpose. The samples were introduced in the split less mode with an injection temperature of 280°C. The transfer line and ion source temperatures were 300°C and 200°C. The column temperature was initially held at 60°C for 5 min, and then raised to 180°C at the rate of 25°C per minutes, then to 220°C at the rate of 10°C per minutes, and finally to 300°C at the rate of 5°C per minutes, held at final temperature for 15 min. Detector temperature was kept at 320°C. The carrier gas was helium, which was used at a constant flow rate of 1 mL per minutes.

Molecular analysis

The Quick-gDNA™ Blood MiniPrep is a simple procedure for the rapid isolation of total DNA (e.g., genomic, mitochondrial, viral) from a variety of biological sample sources. This Procedure has been optimized for maximal recovery of ultra-pure DNA without RNA contamination and is compatible with whole blood (fresh or stored), serum, and plasma. For processing, simply the specially formulated Genomic Lysis Buffer was added to a sample, vortex, and transfer the mixture to the supplied Zymo-Spin™ Column. There is no need for organic denaturants or proteinase K digestion because of the unique chemistries featured in the kit. Instead, the product features Fast-Spin technology to yield high-quality, purified DNA in just minutes. PCR inhibitors are effectively removed during the purification process. DNA purified using the QuickgDNA™ Blood MiniPrep is suitable for PCR, nucleotide blotting, DNA sequencing, restriction endonuclease digestion, bi sulfite conversion/methylation analysis, and other downstream applications.

1- SNP Real Time Taq Man PCR

Applied Biosystems™ TaqMan® SNP Genotyping Assays use TaqMan® 5´ nuclease chemistry for amplifying and detecting specific polymorphisms in purified genomic DNA samples. Each assay allows genotyping of individuals for SNP.

2- Gene expression of hOGG1

Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product that enables it to produce end products, protein or non-coding RNA, and ultimately affect a phenotype, as the final effect. These products are often proteins, but in non-protein-coding genes such as transfer RNA (tRNA) and small

nuclear RNA (snRNA), the product is a functional non-coding RNA.
RNA extraction kit supplied by Direct-zol™ RNA MiniPrep, R2051, ZYMO RESEARCH / USA. RNA was extracted from whole blood sample. A fragment 89 bp of hOGG1 was amplified using a forward and reverse primers were supplied, by IDT (Integrated DNA Technologies company, Canada) table (1).

Table 1. Specific primer for hOGG1gene

Primer	Sequence	Tm (°C)	GC (%)	Products size
Forward	5'-GAAATAGGGAAGGTTGTTAAATAGTAT-'3	51.9	32.1	89
Reverse	5'-AAACTAAAATACGATACCCCATAC -'3	52.8	36.0	

Prime Script TMRT reagent Kit was prepared to carry out the reverse transcription optimized for RT-PCR. It uses Prime Script TM RTase, which has excellent extendibility and allows for efficient, fast cDNA template synthesis for RT PCR. The step experimental procedure is straightforward and well-suited to high-throughput analysis. This kit can be used in combination with Real Time PCR reagent, SYBR (sybr green) Premix. The gene expression level for the sample have been determined using the following equation (obtained from the kit leaflet of Direct-zol™ RNA MiniPrep, R2051, ZYMO RESEARCH / USA).:

ΔCt of patient=Ct target – Ct reference
ΔCt of control=Ct target – Ct reference
ΔΔCt= ΔCt of patient- ΔCt of control

$$\text{Folding}=2^{-(\Delta\Delta Ct)}$$

Results

The participant ages shown in table (2); age of participants ranged from (25-59) yr, they were divided into 4 groups at a range of every ten years, there is no significant difference in age among studied groups

Table 2. The age of participants

Age group	Control	Field	Office	p value
25-35	30	30	29	0.071
36-45	6	5	4	
46-55	10	9	11	
>56	10	12	12	
Total	56	56	56	

PAHs were detected in the blood of field and office groups and not detected in the blood of control group; the level of PAH was significantly higher in field group compared with office group.
HOGG1 gene expression levels were significantly higher in field group than in office group and control group respectively as shown in table (3).
The genotype GG was higher in control groups than office and field groups respectively, where the CC genotype was higher in field than office and control groups respectively. As shown in tables (4 and 5).

Table 3. Results of PAHs and hOGG1 expression among the studied groups

Parameter	Group	Mean±SD	p value
PAH (ppm)	Control	-	0.001
	Office	2.66±0.15	
	Field	6.78±0.27	
HOGG gene expression(fold)	Control	1.1±0.23	0.001
	Office	1.69±0.31	
	Field	4.1±0.46	

Table 4. Frequencies association between OGG1 rs1052133 genotypes in samples of the field and control

		Control n = 56 n (%)	**Field n = 56 n (%)**	**P value**	**Odds Ratio**	**95% CI**
Genotype	GG	17 (8.9%)	5 (5.3%)	0.008	0.22	0.1
	GC	19 (33.9%)	18 (32.1%)	0.922	0.92	0.42 to 2.01
	CC	20 (57.1%)	33 (65.5%)	0.02	2.5	1.21 to 5.50
Allele	G	53 (25.9%)	28 (21.4%)	0.05	0.73	0.42 to 1.44
	C	59 (74.1%)	84(78.5%)			

Table 5. Frequencies association between OGG1 rs1052133 genotypes in samples office and control

		Control n = 56 n (%)	**Field n = 56 n (%)**	**P value**	**Odds Ratio**	**95% CI**
Genotype	GG	17 (30.5%)	10 (17.8%)	0.267	0.45	0.71 to 6.90
	GC	19 (33.9%)	25 (44.6%)	0.333	0.85	0.74 to 3.35
	CC	20 (35.9%)	21 (37.5%)	0.050	2.2	0.21 to 0.95
Allele	G	53 (25.9%)	45 (40.2%)	0.033	1.92	1.09 to 3.38
	C	59 (74.1%)	67 (59.8%)			

The effect of genotype on the level of hOGG1 gene expression are illustrated in figure (1). The results showed higher level of gene expression for the infield samples are showed by the GG genotype (4.7) followed by the CC genotype (4.101) and then GC genotype (3.842). The expression level around the office subjects is showed higher level by the GC genotype (2.368) followed by the CC genotype (1.472) then by GG genotype (0.193).

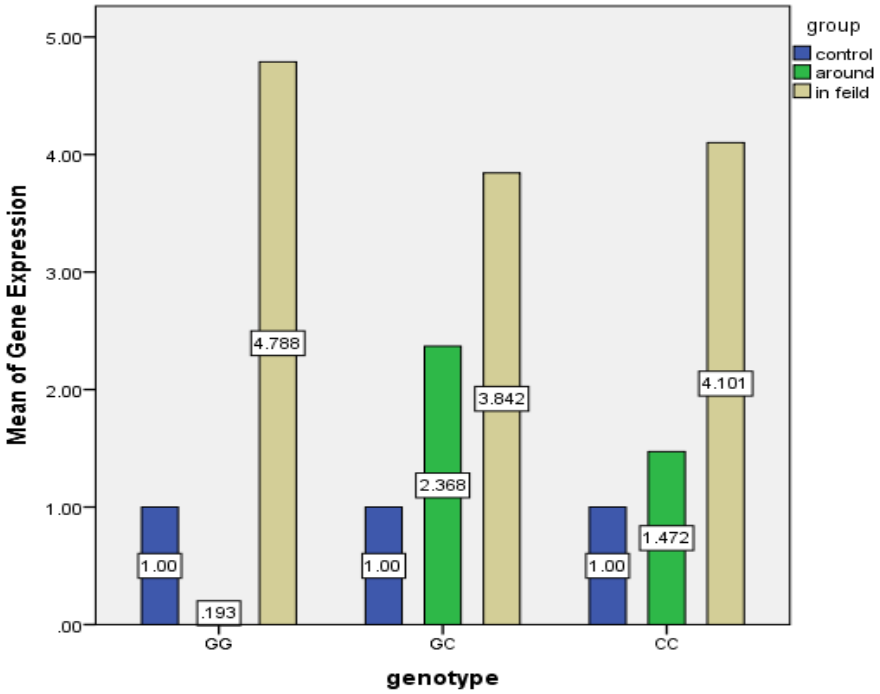

Figure 1. Effect of rs1052133 genotype on hOGG1 gene expression level

Discussion

PAH were not detected in the blood of control group, and their levels were significantly higher in field workers compared with office workers. hOGG1 gene expressions are increased in refineries workers (around field and in field) as compared with control group with significant increase in field workers compared to office workers. Most PAHs are not genotoxic by themselves and must be metabolized to their diol epoxides diene and/or hydroxyl, which then react with DNA to induce genotoxic damage. Genotoxicity plays an important role in the carcinogenicity process and may be in some forms of developmental toxicity [13].The level of hOGG1 was higher in participant with GG genotype than those CC and GC genotype this indicate that GG genotype is a protective genotype in refineries workers against 8-oxo DG accumulation that generate from PAH metabolites in human and CC genotype group the least susceptibility to increase hOGG1 gene expression and the BER pathway eliminates many varieties caused by ROS generate from PAH metabolism. Our results are in line with Hassan results who proposed that GG genotype were significantly correlated with refineries worker's. This indicates that hOGG1 rs1052133 genotypes (CC and CG) promoted the increase 8-OXO-Dg level, which is in contrast to the GG genotype. This suggests that CC genotypes are significantly correlated with the risk of gene variation and mutation. These findings indicate that specific genotypes within a single repair pathway are factors that affect the risk of mutation. Because SNP rs1052133 significantly interacts with numerous variants of proteins with a slightly accelerated or reduced useful activity [14]. These results are in line with Alanazi et al. suggested in his study that that hOGG1 rs1052133 genotypes (CC and CG) promoted the development of leukemia, which is in contrast to the GG genotype. CC genotypes are significantly correlated with the risk of leukemia. These findings indicate that specific genotypes within a single repair pathway are factors that affect the risk of leukemia [15].

In conclusion, refineries workers are at high risk of developing malignant transformation

and serious disease due to high level of PAH compared to other population. SNP in the gene involved in DNA base excision repair hOGG1 rs1052133h might play a crucial role in DNA repair, GG genotype has the most defensive effect Against DNA mutation.

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Author contribution

Al-Ani went to Midland Refineries Company, AL-Daura Refinery and collected blood samples from office and field groups. Blood samples of control group (lives in country sides (rural)) away from Baghdad city) were collected by Shukur.

Al-Ani and Dr. Al-Wasiti took the collected blood samples to Wahj Al-Dna laboratory and Environmental Research Center, Ministry of Science and Technology to observed the process of obtaining the results. All authors collected articles with similar topics.

Conflict of interest

Authors declare they have no conflict of interest with others.

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The Use of Local Anesthesia for Relief of Postoperative Pain After Laparoscopic Cholecystectomy

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Abstract

Background The postoperative pain remains the most prevalent complaint after laparoscopic cholecystectomy for cholelithiasis, which may lead to prolong hospital stay.

Objective To evaluate the effect of wound infiltration and intraperitoneal instillation of Bupivacaine for relief the postoperative pain (within first 24 hours) in patient undergoing laparoscopic cholecystectomy.

Methods A randomized study included 60 patients having symptomatic gallstones attending Al-Yarmouk Teaching Hospital, Department of Surgery, Baghdad, Iraq, during the period from December 2018 to December 2019; for elective laparoscopic cholecystectomy. Patients were divided into two equal groups; in group (A), intraperitoneal 10 ml bupivacaine (0.5%) was given for postoperative pain relief, while group (B) was not given this local anesthesia. The postoperative pain was assessed by Visual Analogue Scale score at fixed time intervals starting 1, 2, 4, 6 hrs then 12, 18 and 24 hrs postoperatively.

Results In group A, 33.3% (n=10) of patients had abdominal pain that needed additional analgesia, while 66.6% (n= 20) of patients, they didn't need additional analgesia. In group B, 93.3% (n=28) of patients had pain and needed additional analgesia, whereas 6.6% (n=2) of patients, they didn't need additional analgesia. The incidence of shoulder pain was 10% (n=3) in group A and 20% (n=6) in group B.

Conclusion The wound infiltration and intraperitoneal instillation of local anesthesia significantly reduces abdominal pain (first 12 hrs), also reduces the need for post-operative additional analgesia, while local anesthesia had no significant effect on shoulder tip pain, post operatively.

Keywords Laparoscopic cholecystectomy, local anesthesia, post operative pain

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List of abbreviations: ASA = American Society of Anesthesiologist, IPLA = Intraperitoneal local anesthetic, LA = Local anesthesia, LC = Local cholecystectomy, VAS = Visual analogue score

Introduction

Laparoscopic cholecystectomy (LC) is considered the standard technique to remove symptomatic gall bladder [(1)]. The pain, which happened after this technique is less and shorter than that caused by open cholecystectomy [(2,3)].

Opioids and non-steroidal anti-inflammatory drugs (NSAIDs) are generally used for treating the post-operative pain after LC with variable success [(4)]. The advantage of infiltration of wounds with local anesthetics (LA), their intraperitoneal instillation as well as the choice and dosages of LA remain controversial [(5)].

Time and pattern of pain after LC on the day of surgery is typically a diffuse abdominal pain, a more so to the right upper quadrant and right shoulder tip [(6,7)]. The pain after laparoscopy is associated with persistent pneumoperitoneum,

sometimes for 3 days, there is significant correlation between gas volume and pain scores [8].

A number of studies reported various treatment modalities to relieve pain after LC. A therapeutic approach using intraperitoneal local anesthetic (IPLA) is remarkable because the beneficial effect of this strategy is closely linked to pain characteristics after LC, which primarily arises from pneumoperitoneum [9]. Bupivacaine is a potent LA with unique characteristics from the amide group of LA.

LA are used in regional anesthesia, epidural anesthesia, spinal anesthesia, and local infiltration. LA generally block the generation of the action potential in nerve cells by increasing the threshold for electrical excitation [10]. Bupivacaine has been demonstrated to produce longer peripheral neural blockade with duration of (6–12 hrs) than lidocaine (1-2 hrs) as such bupivacaine is commonly used for long-acting anesthetic effects, with onset of action within 15 mints and the safe dose is 0.25% mg/kg with maximum dose 2 mg/kg if used without adrenaline [11,12].

This study aimed to evaluate the effect of wound infiltration and intraperitoneal instillation of Bupivacaine for relief the postoperative pain (within first 24 hours) in patient undergoing LC.

Methods

This is a prospective, randomized study conducted in the Department of General Surgery at Al-Yarmouk Teaching Hospital, during the period from December 2018 to December 2019. Patients underwent elective LC for symptomatic cholelithiasis were included in this study.

Seventy-five (75) was the total number of patients, fifteen (15) patients were excluded and only sixty (60) patients were included in this study. All of them belong to American Society of anesthesiologist (ASA) class 1 or 2 (Table 1).

Table 1. American Society of anesthesiologist (ASA) classification of patients' physical pain

ASA class	Physical status
1	Normal healthy patient
2	Patient with mild systemic disease
3	Patient with severe systemic disease
4	Patient with severe systemic disease that is a constant threat to life
5	Moribund patient not expected to survive without emergent procedure

All the data were recorded including (age, gender, weight of the patients, details of procedure, site of pain, CO_2 pressure, duration) and post-operative assessment (pain score, post-operative pain) and the use of visual analogue score (VAS) was explained to the patients, and informed consent was obtained before operation.

Inclusion criteria:

All patients with symptomatic cholelithiasis

Exclusion criteria

Patients unable to understand the VAS (7 patients), conversion to open surgery (5 patients), or patients having CBD stones (3 patients).

Patients were divided into two groups, each of group with 30 patients.

The operation started under general anesthesia with endotracheal intubation using Thiopentone, Halothane-Succinylcholine (muscle relaxant) and Attracurium (as this drug was the only available one and used by the anesthetist during that period of data collection). Pneumoperitoneum was produced by insufflation of CO_2 using Verres needle method (closed technique). The intraperitoneal pressure maintained between 12-14 mmHg. Classical four ports were done. Patients was placed in reverse Trendelenburg and tilt slightly to the left. We used one ampule of Bupivacaine hydrochloride (0.5%) contain 20 ml (5 mg/ml), which equal to 100 mg, we took 10 ml from the ampule of bupivacaine (0.50%) diluted in 100 ml of (0.9%) Normal saline and installed in the sub diaphragmatic and sub hepatic spaces under vision using the sucker.

The other 10 ml from the ampule of Bupivacaine (0.5%) without dilution were used for wounds infiltration and divided as follows; 4 ml for epigastric (10 mm) port site wound at first, then we change the camera from the umbilical to epigastric port, then 4 ml LA for umbilical (10 mm) camera site port wound, and 1 ml for each of the two (5 mm) ports sites wounds.

Time of patient's arrival to the ward (postoperatively) was considered as Zero hr where all the patients were given one Paracetamol vial 1000 mg in this hour. Then the pain intensity was measured at fixed time intervals starting 1, 2 ,4, 6 hrs then 12, 18 and 24 hrs post operatively and was recorded by resident doctors using VAS, also they recorded the number and type of additional analgesic injections that the patient was needed with special forma.

Data analyzed using statistical package for social sciences (SPSS) version 25. The data presented as mean, standard deviation or frequencies and percentages. Independent t-test (two tailed) and Chi square tests were used. P value less than 0.05 was considered significant.

Results

The total number of study participants was 60. All of them had LC for symptomatic cholelithiasis. They were divided into two groups: LA group included 30 patients received LA (as wound infiltration and intraperitoneal instillation) to relieve abdominal pain and control group included the other 30 patients who didn't receive LA.

General characteristics

The distribution of study groups by general characteristics (age and gender) is shown in figure (1).

Study patients' age was ranging from 22 to 68 years with a mean of 42.36 years and standard deviation (SD) of ± 12.04 years. The highest proportion of study patients in LA group was aged <40 years and between 40-59 years (43.3% in both) and in control group was aged between 40 - 59 years (46.7%).

Regarding gender, proportion of females was much higher than males in LA and control groups (86.7%, 83.3% respectively).

Concerning BMI (body mass index) level, the highest proportion of study patients in LA and control groups was overweighed (80% for both).

Surgical information

It was noticed that the highest proportion of study patients needed more than 60 min for duration of surgery in LA and control groups (70% versus 76.7% respectively). Regarding CO_2 pressure, 50% of LA and control groups had their surgery with CO_2 pressure of 12 mmHg (Figure 2).

VAS for abdominal pain

The comparison in means of VAS score of pain between study groups postoperatively at abdominal level are shown in table (2). The means of VAS score after 1, 2, 4, 6, and 12 hrs. after operation were significantly lower in LA group than that in control group (1.63 versus 2.46, P= 0.002; 1.5 versus 2.76, P= 0.001; 1.6 versus 2.8, P= 0.001; 2.0 versus 4.36, P= 0.001; and 2.33 versus 4.46, P= 0.001 respectively).

No statistically significant differences (P ≥0.05) in mean of VAS score between study groups at abdomen level after 18 and 24 hrs.

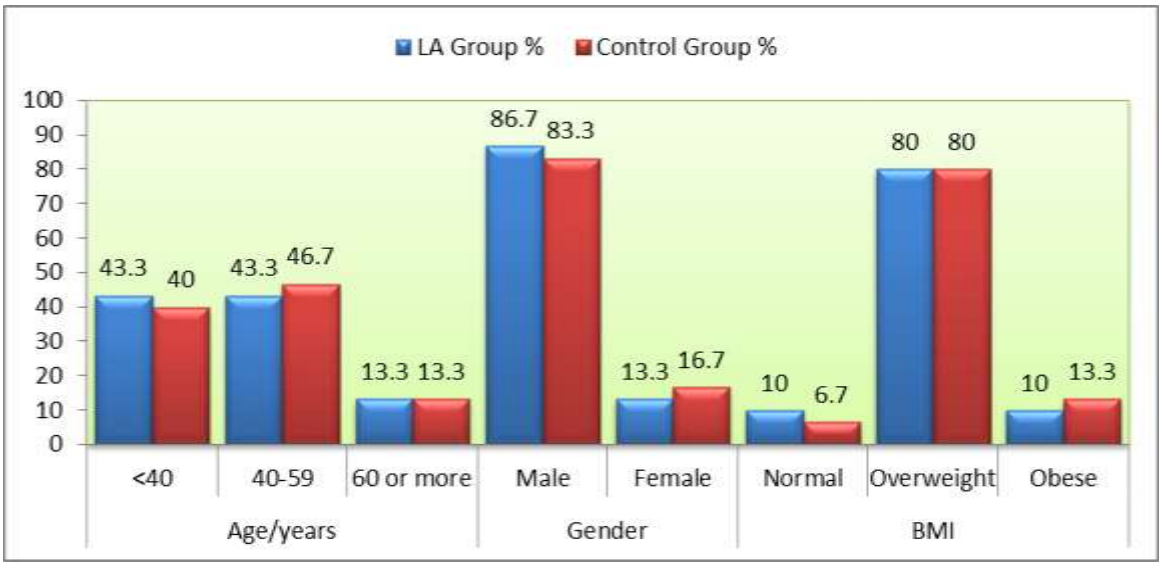

Figure 1. Distribution of study groups by age, gender and body mass index

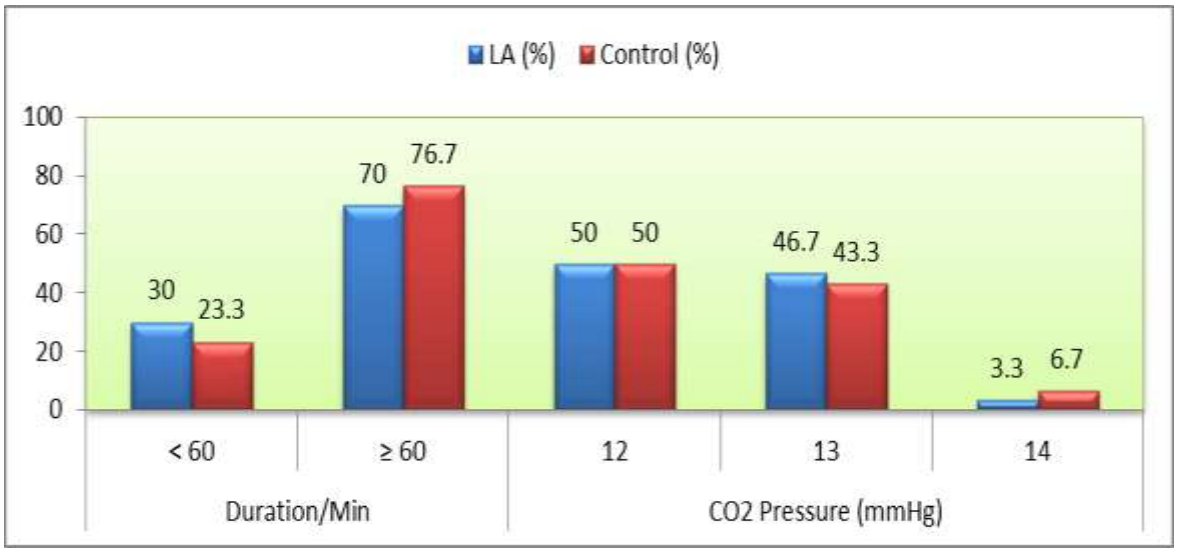

Figure 2. Distribution of study groups by surgical information

Table 2. Comparison in means of VAS score of pain between study groups postoperatively at abdominal level

Time	VAS Score for pain		P Value
	LA group Mean±SD	Control group Mean±SD	
After 1 hr	1.63±0.61	2.46±1.27	0.002
After 2 hrs	1.5±0.5	2.76±1.71	0.001
After 4 hrs	1.6±0.67	2.8±1.58	0.001
After 6 hrs	2.0±0.9	4.36±2.55	0.001
After 12 hrs	2.33±1.12	4.46±2.78	0.001
After 18 hrs	2.83±1.6	3.73±2.24	0.079
After 24 hrs	2.5±0.9	2.86±0.8	0.089

VAS for shoulder tip pain

Table 3 shows the comparison between study group according to shoulder tip pain after operation. No statistically significant differences (P ≥0.05) between study groups regarding shoulder tip pain after operation.

Table 3. Comparison between study group according to shoulder tip pain after operation

Shoulder tip pain		Study group		Total (%) n= 60	P Value
		LA (%) n= 30	Control (%) n= 30		
After 1 hr	Yes	0 (0.0)	1 (3.3)	1 (1.7)	0.313
	No	30 (100)	29 (96.7)	59 (98.3)	
After 2 hrs	Yes	0 (0.0)	0 (0.0)	0 (0.0)	1.000
	No	30 (100)	30 (100)	60 (100)	
After 4 hrs	Yes	0 (0.0)	0 (0.0)	0 (0.0)	1.000
	No	30 (100)	30 (100)	60 (100)	
After 6 hrs	Yes	0 (0.0)	0 (0.0)	0 (0.0)	1.000
	No	30 (100)	30 (100)	60 (100)	
After 12 hrs	Yes	1 (3.3)	0 (0.0)	1 (1.7)	0.313
	No	29 (96.7)	30 (100)	59 (98.3)	
After 18 hrs	Yes	0 (0.0)	2 (6.7)	2 (3.3)	0.150
	No	30 (100)	28 (93.3)	58 (96.7)	
After 24 hrs	Yes	2 (6.7)	5 (16.6)	7 (11.7)	0.228
	No	28 (93.3)	25 (83.4)	53 (88.3)	

Use of additional analgesia

The association between study group and using of additional analgesia is shown in table (4). In this study, 90.9% of patients who didn't need additional anesthesia were received local anesthesia (LA group) with a significant association (P= 0.001) between study group and using of additional analgesia.

Table 4. Association between study group and using of additional analgesia

Use of additional analgesia	Study group		Total (%) n= 60	P - Value
	LA (%) n= 30	Control (%) n= 30		
Yes	10 (26.3)	28 (73.3)	38 (63.3)	0.001
No	20 (90.9)	2 (9.1)	22 (36.7)	

VAS score of abdominal pain according to duration of surgery

<60 min

The comparison in means of VAS score of pain between study groups in patients with duration of surgery less than 60 mins. is shown in table (5).

There were no significant differences (P ≥0.05) between study groups in means of VAS score of pain in patients with duration of surgery less than 60 min in all times after operation.

≥60 min

The comparison in means of VAS score of pain between study groups in patients with duration of surgery ≥60 min is shown in table (5). Means of VAS score of pain after 1, 2, 4, 6, and 12 hrs were significantly lower in LA group than that in control group (1.61 versus 2.56, P= 0.006; 1.52 versus 2.73, P= 0.002; 1.52 versus 2.86, P= 0.001; 1.85 versus 4.39, P= 0.001; and 2.33 versus 5.0, P= 0.001 respectively).

There were no significant associations (P ≥0.05) between study group in means of VAS score of pain in patients with duration of surgery ≥60 min after 12 and 24 hrs postoperatively.

Table 5. Comparison in means of VAS score of pain between study groups in patients with duration of surgery < 60 min and ≥60 min

Duration of surgery	VAS Score for pain in patients with duration of surgery <60 min			VAS Score for pain in patients with duration of surgery ≥60 min		
	LA Mean±SD	Control Mean±SD	P value	LA Mean±SD	Control Mean±SD	P value
After 1 hr	1.66±0.7	2.14±0.89	0.255	1.61±0.58	2.56±1.37	0.006
After 2 hrs	1.44±0.52	2.85±2.03	0.063	1.52±0.51	2.73±1.65	0.002
After 4 hrs	1.77±0.97	2.57±1.13	0.154	1.52±0.51	2.86±1.71	0.001
After 6 hrs	2.33±1.32	4.28±2.62	0.072	1.85±0.65	4.39±2.58	0.001
After 12 hrs	1.77±0.44	2.71±1.25	0.055	2.33±1.42	5.0±2.92	0.001
After 18 hrs	2.44±1.58	3.71±2.05	0.185	2.76±1.75	3.73±2.33	0.128
After 24 hrs	2.44±1.5	3.65±0.89	0.067	2.85±0.57	3.39±1.43	0.252

Discussion

Early pain after LC is a complex process and includes different pain component secondary to different pain mechanisms, such as surgical trauma secondary to gall bladder removal, abdominal distention, and pneumoperitoneum using CO_2 [13]. Adequate early postoperative pain relief after LC is an essential goal to help the patient discharge home early with minimum pain and in stable condition [14].

In this study, intraperitoneal instillation had been done and ports sites infiltration with Bupivacaine (0.5%) had been ensured, which found to be useful in reducing the intensity of

pain after LC and there was significant difference in the total amount of analgesia required between the two groups (LA group compared control group). The current results are in agreement with a study by Al kazwini in 2017 [15], which concluded that intraperitoneal installation was effective in reducing immediate postoperative pain following LC, while the study conducted by Hosseini et al. in 2013 showed that intraperitoneal administration of Lidocaine 200 ml after elective LC has no considerable effect on the abdominal and scapular pain [16]. But it is disagreeing with Gluck et al. randomized controlled trial in 2021, who found that the level of postoperative pain, either at rest or with change of position, was not significantly different between the groups, at all-time points. Application of subcutaneous and/or intraperitoneal analgesia is not effective in reducing pain after operative laparoscopy [17].

The current study showed that intraperitoneal instillation of LA had little effect on postoperative shoulder pain and this was consistent with the findings of other study done by Cunningham et al. in 2020, in which there was a significant reduction in shoulder-tip pain scores in the Levobupivacaine group at 3 hrs, then a significant reduction in wound-pain scores in the Levobupivacaine group at 8 hrs (p=0.04) and at day 4 postoperative [18]. This variation in results could be due to the variation and effects of different factors like in adverse event profile, dosing and toxicity, pharmacodynamics, pertinent for members of the inter professional team for the treatment of patients when local anesthesia is warranted [19].

Our study showed that the analgesic requirements is less in LA group compared with control group. This supported by other study Yeh et al. in 2014, in which combined wound and intraperitoneal LA use after LC significantly decreased the immediate postoperative pain and may explain the reduced use of Meperidine and earlier discharge of patients so treated [20].

In conclusion, the use of Bupivacaine in the intraperitoneal instillation and wound infiltration significantly reduces postoperative abdominal pain after LC in first 12 hrs, also reduces requirements for additional analgesia especially in those whose surgery lasts ≥60 min. While intraperitoneal subdiaphragmatic instillation of Bupivacaine was not effective in reducing shoulder pain, in the early postoperative period.

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Author contribution

Dr. Mallallah: Data collection, writing and analysis. Dr. Al-Mafrachi: Study design and supervision.

Conflict of interest

None.

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Association between Vascular Endothelial Growth Factor Gene Polymorphisms and the Risk of Preeclampsia in Iraqi Pregnant Women

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Abstract

Background Vascular endothelial growth factor (VEGF) is an essential factor for angiogenesis and plays important role in placental development.

Objective To investigate the association between VEGF single nucleotide polymorphisms (SNPs) +936 Cytosine/Thymine (C/T) and -634 Guanine/Cytosine (G/C) and preeclampsia risk.

Methods A total of 50 cases of pregnant women with preeclampsia and 50 healthy pregnant women (as a control) were involved in this case control study. Blood samples were collected form each woman and the Deoxyribonucleic acid (DNA) was extracted. Amplification of VEGF gene was done by conventional polymerase chain reaction (PCR) and then detection of SNPs-634 G/C and +936 C/T were carried out by restriction fragment length polymorphism PCR (PCR-RFLP).

Results The frequency of different genotypes of VEGF SNPs +936 C/T &-634 G/C are in accordance with Hardy Weinberg equilibrium. VEGF polymorphism +936 C/T appeared in 3 genotypes after digestion with restriction enzymes Cytosine Cytosine (CC), Cytosine Thymine (C/T) & Thymine Thymine (TT). VEGF SNP -634 G/C appeared in 3 genotypes after digestion with restriction enzymes; those were Guanine Guanine (GG), Guanine Cytosine (GC) and CC. The heterozygous genotype CT of polymorphism +936 C/T was more frequent among preeclamptic patients than the controls (26% versus 16%). Likewise, TT genotype was more frequent among preeclamptic patients (8% versus 2%) with no significant differences. At the allelic level, the difference was more prominent. The frequency of mutant allele (T allele) was much more frequent in preeclamptic patients than controls (21% versus 10%) with a statistically significant difference (p=0.049). Although CC genotype of polymorphism -634 G/C was more frequent among preeclamptic patients than controls (14% versus 10%), the difference was not significant (p=0.704). Likewise, there were no significant differences in allele frequency.

Conclusion The study suggested that the mutant T allele of VEGF +936 C/T polymorphism was associated with increased preeclampsia risk and disease development.

Keywords Vascular endothelial growth factor, polymorphisms, preeclampsia

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List of abbreviations: bp = Base pair, C = Cytosine, DBP = Diastolic blood pressure, DNA = Deoxy ribonucleic acid, G = Guanine, HWE = Hardy-Weinperg equilibrium, Kb = Kilo Base, P = Short arm of a chromosome, PCR = Polymerase chain reaction, PE = Preeclampsia, PIGF = Placental growth factor, PIH = Pregnancy induced hypertension, RFLP = Restriction fragment length polymorphism, SBP = Systolic blood pressure, SNP = Single nucleotide polymorphism, T = Thymine, VEGF = Vascular endothelial growth factor

Introduction

Preeclampsia (PE) has been recognized as a multifactorial disorder with great phenotypic diversity and it is caused by a complex interplay between genetic and

environmental factors [1]. PE is a leading cause of maternal death. The World Health Organization (WHO) estimates that between 50,000 and 75,000 women die globally of this condition annually [2].

PE is diagnosed as a systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg on 2 occasions at least 4 hours apart after 20 weeks of gestation in a previously normotensive patient with the new onset of any of the following (with or without proteinuria): platelet count <100,000/µl, serum creatinine >1.1 mg/dl or doubling of the creatinine concentration in the absence of other renal disease, liver transaminases at least twice the upper limit of the normal concentrations for the local laboratory, pulmonary edema and cerebral or visual symptoms (e. g new onset and persistent headache, blurred vision, flashing lights or sparks, scotomata) [3].

Vascular endothelial growth factor (VEGF) is an essential factor for angiogenesis and plays important role in placental development [4].

VEGF gene is located on the chromosome 6 p21.3, the full length is 28kb, and encoding gene length is 14kb, consisting of 8 exons and 7 introns. The coding product is two bond linked glycoproteins, due to different splicing site of the gene, 7subtypes of the protein can be found [5]. VEGF (or VEGF-A) belongs to a gene family of those placental growth factor (PlGF), VEGF-B, VEGF-C and VEGF-D. They share structural features typical of the VEGF family, but display different biological activities, mainly owing to their different specificities for the known VEGF receptors [6].

VEGF gene polymorphisms play an important role in regulating and altering the protein expression and function, and changing the susceptibility to PE [7]. Many polymorphisms of the VEGF gene have been identified so far. Few of them have been correlated with variation in VEGF protein production [8]. Specifically, C936T located in the 39-untranslated region and -634 G/C in the 59-untranslated region [9]. The single nucleotide polymorphisms (SNP), rather than the gene, is the currency of large scale, high-throughput studies of the human genome, which are the key to complex diseases like PE [10].

The objectives of this study were to investigate the association between VEGF SNPs +936 Cytosine/Thymine (C/T) and -634 Guanine/Cytosine (G/C) and preeclampsia risk.

Methods

A case-control study was conducted at Al-Imamein Al- Kadhimein Medical City for the period from February 2019 to the end of October 2019. A total of 100 pregnant women with gestational age ≥20th weeks were included in this study. They were informed about the nature of the study and verbal with written consents were obtained from them. Eligible women were divided in to two equal groups, preeclampsia group (PG) included 50 pregnant women who had been diagnosed with PE and a control group (C) included 50 pregnant women who were normotensive with no symptoms or signs of PE & no proteinuria. Women with chronic hypertension, diabetes (type 1 or 2), chronic renal disease, autoimmune disease and rheumatic disease were excluded from the study.

DNA extraction and genotyping

Five ml of venous blood were collected from each participant. DNA was isolated from 200 µl of anticoagulated peripheral blood using a commercially available kit according to the manufacturer's instructions (QIAamp DNA Blood Mini Kit; Qiagen Inc., USA). Amplification of the two regions of the VEGF gene containing the polymorphisms-634G/C and +936C/T were carried out in a Master cycler gradient (Hybaid/UK) thermal cycler in 20 µl reaction volumes containing 20 mmol/l Tris-HCl (pH 8. 4), $MgCl_2$, 50 mmol/l KCl, 0.2 mmol/l of each nucleotide, 20 pmol of each of the forward and reverse primers, 1 U Platinum Taq polymerase (Pioneer, Korea) and 500 ng of DNA. Following an initial denaturation step (5 min at 94°C), samples were subjected to 35 rounds of polymerase chain reaction (PCR) consisting of

94°C for 40 s, 58°C (-634G/C) or 64°C (+936C/T) for 1 min; and 72°C for 40 s with a final extension time of 5 min at 72°C. For the -34G/C the following primers amplified a fragment of 304 bp: forward 5'-ATTTATTTTTGTCTGTCTGTCTGTCCGTCA-3' and for the +936C/T the following primers amplified a fragment of 208 bp: forward 5' AAGGAA GA GGAG ACTCTGCGCAGAGC-3', reverse 5' TAAATGTATGTATGTGGGTGGGTGTG TCTACAGG-3'. The VEGF -634G/C polymorphism was analyzed by digestion of the PCR product with restriction endonuclease BsmfI (New England Biolabs, USA).

The -634G allele was cut into two fragments of 193 and 111 bp while the -634C allele remained uncut (304 bp). The VEGF 936C/T polymorphism was analyzed by digestion of the PCR product with restriction endonuclease NlaIII (New England Biolabs). The 936C allele remained uncut (208 bp), while the 936T was cut into two fragments of 122 and 86 bp.

Statistical Analysis

The statistical package for the social sciences (SPSS, version 20) was used for statistical analysis. Continuous variables were expressed as mean±standard deviation (SD). Risk association between the different genotypes of VEGF gene polymorphisms and PE susceptibility was estimated by the calculation of odd ratio (OR) and 95% confidence intervals (CI) using binary logistic regression. For this analysis, women who were homogenous for the wild genotype were considered as a reference, and polymorphisms as dependent variables. Chi square was used for testing the deviation from Hardy-Weinberg equilibrium as well as for comparing categorical variables. A p-value <0.05 was considered statistically significant.

Results

Mean age of PE patients was 26.94±4.13 years, which was slightly higher than the mean age of the controls (25.92±8.09 years) with no significant difference. Women in control group had higher mean values for parity, gestational age, and hemoglobin (Hb) (1.78±0.83, 36.24±5.15 weeks, and 11.58±1.91 g/dl respectively) than PE patients (1.12±0.65, 35.76±6.06 weeks, and10.8±1.1 g/dl, respectively); however, the differences were not significant. On the other hand, positive family history of PE was far more frequent among PE women than controls (36% versus 4%) with a highly significant difference (P value <0.001). In contrast, women in the control group showed significantly higher platelets count than PE women (236.65±58.44×10^3/mL versus 146.86±62.19 ×10^3/mL). Each of the other parameters (albumin in urine, systolic blood pressure and diastolic blood pressure), per se, were significantly higher in PE patients than controls (Table1).

The frequency of different genotypes of both VEGF +936 C/T and VEGF -634 G/C are in accordance with Hardy-Weinberg Equilibrium (HWE).

The distribution of different genotypes and allele of VEGF +936 C/T polymorphism in PE patients and controls is shown in table (2). The heterozygous genotype CT was more frequent among PE patients than control (26% vs. 16%), the difference was not significant (p= 0.16). Likewise, TT genotype was more frequent among PE patients (8% vs. 2%) with no significant difference (p=0.455). At allelic level, the difference was more frequent. The frequency of mutant allele (T allele) was much more frequent in PE patients than controls (21% vs. 10%) with a significant difference (p= 0.049).

Table 1. Demographic, reproductive and clinical characteristics preeclampsia patients and healthy controls

Variables		PE patients N=50	Controls N=50	P value
Age, years (mean ±SD)		26.94±4.13	25.92±8.09	0.236
Parity (mean ±SD)		1.12±0.65	1.78±0.83	0.136
Family history	No	32 (64%)	48 (96%)	<0.001
	Yes	18 (36%)	2 (4%)	
Gestational age (weeks) (mean±SD)		35.76±6.06	36.24±5.15	0.202
Hemoglobin (g/dL) (mean±SD)		10.8±1.1	11.58±1.91	0.072
Platelets count (×10^3/mL) (mean ±SD)		146.86±62.19	236.65±58.44	0.022
Albumin in urine (+) (mean±SD)		2.36±0.81	0.0±0.0	<0.001
SBP (mmHg) (mean ±SD)		158.5±18.23	114.6±9.6	<0.001
DBP (mmHg) (mean±SD)		101.0±12.21	70.0±8.51	<0.001

SBP: systolic blood pressure, DBP: diastolic blood pressure

Table 2. Genotypes and allele frequencies of VEGF +936C/T gene polymorphism in PE patients and controls

VEGF +936C/T		Cases n=50	Control n=50	P-value	OR (95%CI)
Genotype	CC	33 (66%)	41 (82%)	0.169	1.0
	CT	13 (26%)	8 (16%)	0.16	4.97 (0.53-46.62)
	TT	4 (8%)	1 (2%)	0.455	2.46 (0.23-26.11)
	HWE	0.126	0.432		
Allele	C	79 (79%)	90 (90%)	0.049	2.39 (1.06-5.38)
	T	21 (21%)	10 (10%)		

The distribution of different genotypes and allele of VEGF -634 G/C polymorphism showed difference between PE patients and controls, although CC genotype was more frequent among PE patients than controls (14% vs. 10%), the difference was not significant (P=0.704). Likewise, there were no significant differences in allele frequency of this polymorphism between patients and control. The frequency of mutant allele (C allele) in patients and controls was 38% and 32% respectively with a (P=0.46) as shown in table (3).

Discussion

Over the last decade, extensive research has been conducted to better understand the genetic components of the pathophysiology of PE. However, no universally accepted genetic factors can explain the onset and progression of this multifactorial disorder [(11)]. Because VEGF is known to play a role in the regulation of cytotrophoblast invasion and placentation, and there is evidence of abnormal placentation in preeclamptic placenta, it suggested that the genes related to VEGF activity would be a risk factor for PE [(12)].

Table 3. Genotypes and allele frequencies of VEGF-634G/C gene polymorphism in PE patients and controls

VEGF -634G/C		Cases n=50	Control n=50	P-value	OR (95% CI)
Genotype	GG	19 (38%)	23 (46%)	0.671	1.0
	GC	24 (48%)	22 (44%)	0.426	1.7 (0.46-6.2)
	CC	7 (14%)	5 (10%)	0.704	1.28 (0.36-4.64)
	HWE	0.896	0.938		
Allele	G	62 (62%)	68 (68%)	0.46	0.76 (0.42-1.37)
	C	38 (38%)	32 (32%)		

There are several SNPs in the VEGF gene, including +936, -634, -2578 & -1154 positions, these SNPs could alter gene expression and protein production. Representative gene mutations of VEGF such as C-936T and G-634C were associated with decreased levels of circulating VEGF [(4)]. This is suggested to be associated with increased risk of PE [(13)]. The current study evaluated the association between two common functional VEGF polymorphisms SNP +936 C/T and -634 G/C and PE risk; SNPs for which an association with PE were reported in other population and considering the potential impact on gene expression. The current study found that there is a significant association between the frequency of mutant allele (T allele) for the polymorphism +936 C/T and PE patients than controls (21 % vs 10%) (P value 0.049).

Although the genotypes (CT) and (TT) of SNP +936 C/T were more frequent among PE patients but the differences were statistically insignificant. Likewise, the frequency of CC genotype and the mutant allele (allele C) of SNP -634 G/C were higher among PE patients, the differences were not significant. Also, this study found highly significant difference of family history of PE in PE cases compared to the controls (36% vs 4%) (P value <0.001). There are several reports about the association between maternal VEGF polymorphisms and the risk of PE in different countries and ethnic groups with inconsistent results.

Papazoglou et al. involved 42 PE and 73 healthy control of Caucasians ethnicity who were genotyped for -634 G/C and +936 C/T polymorphisms of the VEGF gene. They reported no significant association between genotypic or allelic frequencies. However, with severe PE there was statistically significant difference for allelic frequencies of the +936 C/T polymorphism [(14)].

Kim et al. in a retrospective case control study in Korean pregnant women included PE cases and 237 controls healthy pregnant suggested that there is no significant difference for +936 C/T polymorphism between PE cases and controls. This result is discordant with the findings of the current study; these researchers also investigated SNP -634 G/C and found no significant association between this SNP and PE [(15)].

Garza-Veloz et al. included 78 PE cases and 86 normotensive pregnant controls of Latinos ethnicity. They found no association between VEGF allele, genotype or haplotype frequencies and PE, its severity or onset of the disease [(16)].

Cheng et al. carried a meta-analysis including 11 case-control studies with 1069 PE cases and 1315 controls with different ethnicities indicated that there is significant association between SNP +936 C/T and the risk of PE. Pregnant women carrying the T allele have significantly higher risk of PE than pregnant women carrying the +936 CC genotype [(13)].

Procopciuc et al. found in their study in Romania of 70 PE women and 94 normal pregnant found that the presence of T allele and TT genotype of SNP +936 C/T significantly increases the risk of pregnancy induced hypertension (PIH), mild and severe PE [(17)].

Keshavarzi et al. investigated the association between PE risk and -634 G/C polymorphisms in women of Asian ethnicity, they found that VEGF -634 GC and CC genotypes were significantly higher in PE pregnant women and associated with 2.6 and 2-fold higher risk of PE respectively [18].

The discrepancy between the findings of the current study and the data of some groups for VEGF polymorphisms may be explained by ethnic variation, differences of the VEGF gene polymorphisms in different regions and population, genotyping methods and gene-environmental interaction.

In conclusion, the mutant T allele of VEGF +936 C/T polymorphism was significantly associated with increased PE risk, disease development and could be a susceptibility biomarker for PE, while VEGF -634 G/C polymorphisms had no significant association with PE.

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Author contribution

Dr. khaleel: Scientific work, investigations, writing and editing of all data. Dr. Al-Moayad: Supervision of the study and final editing of manuscript.

Conflict of interest

Authors declare that there is no conflict of interest.

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Treatment Intertrochanteric Fracture of Femur in Elderly by External Fixation: Prospective Case-Series Study; Ibn-Sina Training Hospital, Baghdad

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Abstract

Background Internal fixation is gold standard in treatment of intertrochanteric fractures, but in patients with anesthesia or surgical hazards the external fixation should be explored as a safe approach to reduce operative time and complications risk.

Objective To assess the outcome of external fixation of an intertrochanteric fracture is in elderly patients with co-morbidities.

Methods Twenty-eight elderly patients with non-pathological fractures, closed, who were 65 years old or older, unsuited for surgery for a long period, and had chronic uncontrolled medical problems such as hypertension, diabetes mellitus, or heart disease were included in the study. Criteria for exclusion include reverse obliquity fractures, dementia, pathological fractures and prior hip fractures. Those patients treated with percutaneous external fixation under image intensifier from 2015 to 2020 in Ibn Sina Training Hospital in Baghdad.

Results At 1-year follow-up, 80% returned to pre-fracture ambulatory status. Average time to fixator removal was 12 weeks. There were no cases of pin loosening, breakage, or penetration of femoral head. All patients were evaluated clinically and radiologically for 24 months except 3 patients, 2 died 6 months' post-operative due to causes unrelated to the surgery, and 1 lost follow up 3 months after surgery. Excellent and good results were found in 8 patients. The time of radiological union and fixator removal about 12 weeks. Pin tract infection occurred in most of the patients. Varus malalignment occurred in 20% of patients. Shortening ranged from 0 to 3 cm.

Conclusion External fixation of intertrochanteric fractures in elderly high-risk patients is a dependable, successful, and safe therapeutic option.

Keywords Intertrochanteric fracture, external fixator, elderly patient

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List of abbreviations: None

Introduction

In the elderly, intertrochanteric fractures are most commonly caused by low-energy trauma (such as mild falls). At least 30% of beds in orthopedic institutes are occupied by patients with this type of fracture [1-3]. These fractures are more common in elderly, especially those over 65 years, because bone mass loss (osteoporosis) is much larger [4-5].

The most important goals of treatment for intertrochanteric fractures are to mobilize the patient in a short period of time and guarantee proper union [6]. Surgical procedures are the only way to attain these goals. Internal fixation

is the gold standard treatment for osteoporosis. Implant failures and mal-unions are common in patients treated with internal fixation [5,6].

Many internal fixation devices have been employed, including angulated plates, gamma nails and sliding hip-plates.

Intertrochanteric fractures are particularly common in individuals with poor general health who are unable to endure general anesthesia or who are unable to undertake invasive procedures due to chronic conditions. It is not possible to treat such individuals with long-term immobilization due to the risk of decubitus ulcers, pneumonia, urinary tract infections, deep vein thrombosis, and cardiac problems [5-9].

For the first time in 1943, Anderson et al. used external fixation to treat fractures of the intertrochanteric area [10]. External fixators were developed, and new materials such as hydroxyapatite-coated pins were introduced, prompting surgeons to reconsider external fixators as an alternate option for treating intertrochanteric fractures in older high-risk patients [11].

This study aimed to assess the effectiveness of external fixation in treatment high risk elderly patients with intertrochanteric fracture.

Methods

From March 2015 to March 2020, 28 elderly patients with intertrochanteric fractures were operated on with external fixation and followed up.

There were 15 males and 13 females, with 15 having right side fractures and 13 having left side fractures, 18 had stable fractures and 10 unstable fractures (classified according to intact posteromedial cortex or not). Patients with non-pathological fractures, closed, who were 65 years old or older, unsuited for surgery for a long period, and had chronic uncontrolled medical problems such as hypertension, diabetes mellitus, or heart disease were included in the study.

Criteria for exclusion included reverse obliquity fractures, dementia, pathological fractures and prior hip fractures.

The patients ranged in age from 66 to 81 years old. Traffic accidents caused 8 fractures, while falls caused 20. Following hospitalization, the patients were operated on an average of 3 days (1 to 6 days).

Surgical Procedure

An hour before surgery, intravenous wide spectrum antibiotics were given. Under C arm supervision, the patient was putting in supine position on orthopedic traction table. Anesthesia is administered by spinal or epidural anesthesia or local nerve block.

On the fractured side, reduction was performed by placing the limb into 20°-30° abduction and 10°-15° internal rotation, acceptable reduction depending on restoring the Shenton line by AP view.

The first pin with the proper neck-shaft angle and ante version angle under fluoroscopy was put into the femoral neck through a tiny incision at the base of the greater trochanter, across the fracture site. A couple of pins were installed. The pin heads were spaced 10 mm apart from the joint line. allows the proximal pins to be inserted at a 135° angle to the fixator's stem. Three 5-mm pins were placed into the femur's shaft in the middle third. The frame was tightened and the final position was confirmed (Figure 1).

Antibiotics and analgesics were administered for three days, and anticoagulants were prescribed during the non-weight bearing period. Radiographs in AP and lateral views were taken. The average length of stay in the hospital after surgery was 4 days (range 3-5 days). On the first postoperative day, active hip and knee exercises were begun. On the second or third day, the patients were mobilized with partial weight-bearing using a walker. Patients were required to visit the out-patient clinic every two weeks during the first month, then every month after that for clinical and radiological evaluations until the frame was removed when the patient can fully weight bearing without pain at fracture site.

Figure 1. A and B position of patient in the theatre with c arm. C and D pre and postoperative x-ray

Results

The time of follow-up was 24 months. Only twenty-five of the 28 operated patients (13 females and 12 males) were clinically and radiographically evaluated; two patients died due to causes unrelated to surgery within the first six months and one patient lost to follow-up.

Data about side and mode of fracture were collected and summarized in table 1. Thirteen patients of participants have right side fracture. In 28% of patients the cause was road traffic accidents. Regarding morbidity, the highest percentage has diabetes mellitus 36%.

Table 1. Basic characteristics for participants shown as frequency and percentages

Variable		Frequency	Percentage
Gender	Male	12	48
	Female	13	52
Age distribution (yr)	66-70	12	48
	70-73	6	24
	73-81	7	28
Side of fracture	Right	13	52
	Left	12	48
Mode of fracture	Road traffic accidents	7	28
	Fall on ground	5	20
	Fall from height	8	32
	Others	5	20
Co-morbidities	Diabetes mellitus	9	36
	Ischemic Heart Disease	6	24
	Hypertension	3	12
	Renal Failure	4	16
	Others	3	12

Other data as time interval between injury and surgery, hospital stay, surgery time and time to union are summarized in table 2.

Table 2. Basic characteristics shown as mean with minimum and maximum value

Variable	Mean	Minimum	Maximum
Age (yr)	71.7	66	81
Injury to surgery time (day)	2.72	1	6
Hospital stay (day)	2.6	1	5
Surgery time (minute)	51.8	40	70
Time to union (week)	14.4	10	31

At one year, 18 of 25 followed patients (72%) had regained their normal functional status and were able to walk with the use of a cane.
At the final follow-up, five of the 25 patients (20%) who were not utilizing mobility aids preoperatively required a cane or walker.

Judet's grading system was used to grade the functional results [12]. There were 20 (80%) great results, 4 (16%) acceptable results, 1 (4%) average result and no poor outcomes as shown in (figure 2).

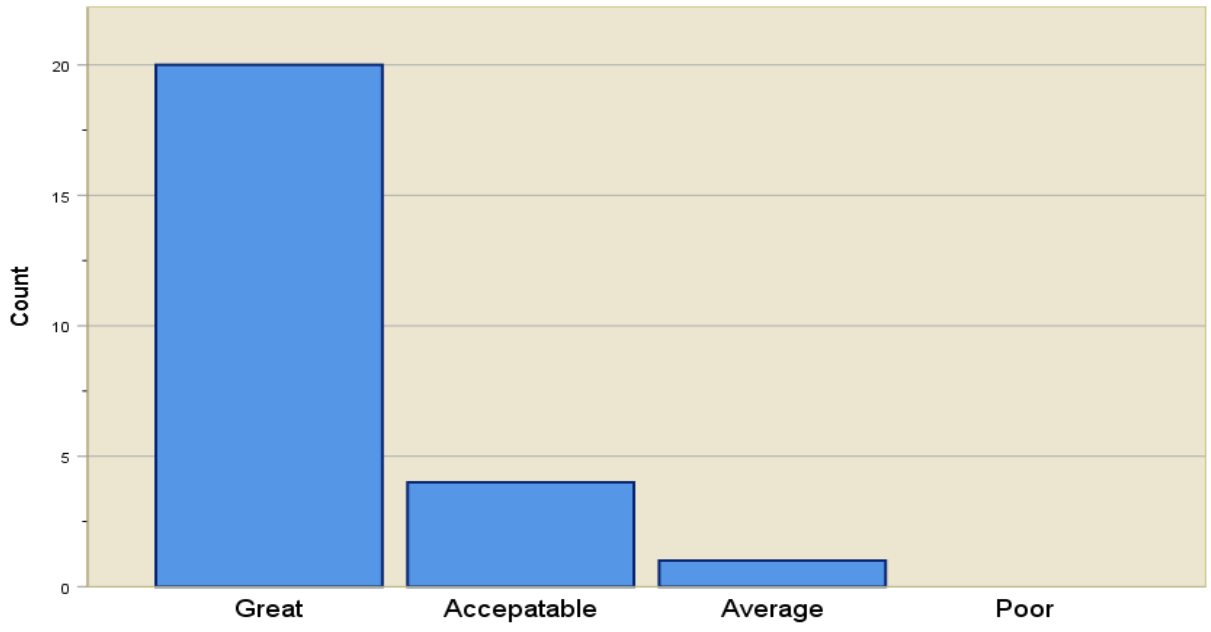

Figure 2. Judet's grading for patient after 24 months of surgery

Five patients (20%) experienced quadriceps muscle wasting, which was reversed after an intensive physiotherapy treatment. Because trochanteric fractures originate through the vascular cancellous bone, non-union is uncommon.

Coxa vara deformity and length disparities between extremities were noted on AP pelvic x-rays. Nine patients had varus malalignment less than 10 degrees, 5 patients had more than 10 degrees, and 11 patients had no varus angulation. Impaction and varus deformity caused shortening in 5 cases ranging from 0 to 3 cm.

The time to begin full weight bearing varied from 6 to 16 weeks, with an average of 12 weeks. In outpatient clinics, the time it took to union and remove the fixator about 10 to 16 weeks (Figure 3).

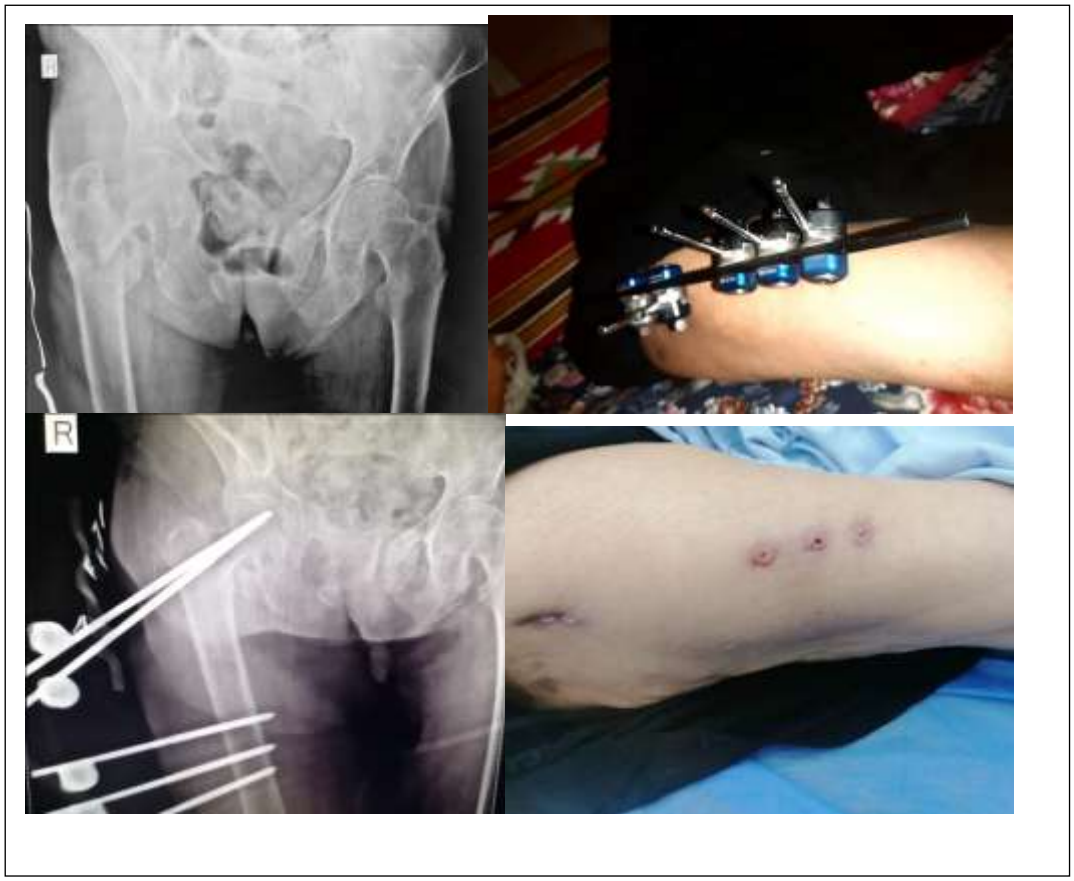

Figure 3. A patient before and after the frame was removed three months after surgery

All patients had pin tract infection to varying degrees, which were treated with antibiotics and daily dressings and were totally cured, with the exception of 5 patients who had moderate pin tract infection, for whom early removal was required to control infection.

There were no instances of pin loosening or femoral head penetration.

The fixator was well accepted, and no patient had any problems sitting or lying down with it (Figure 4).

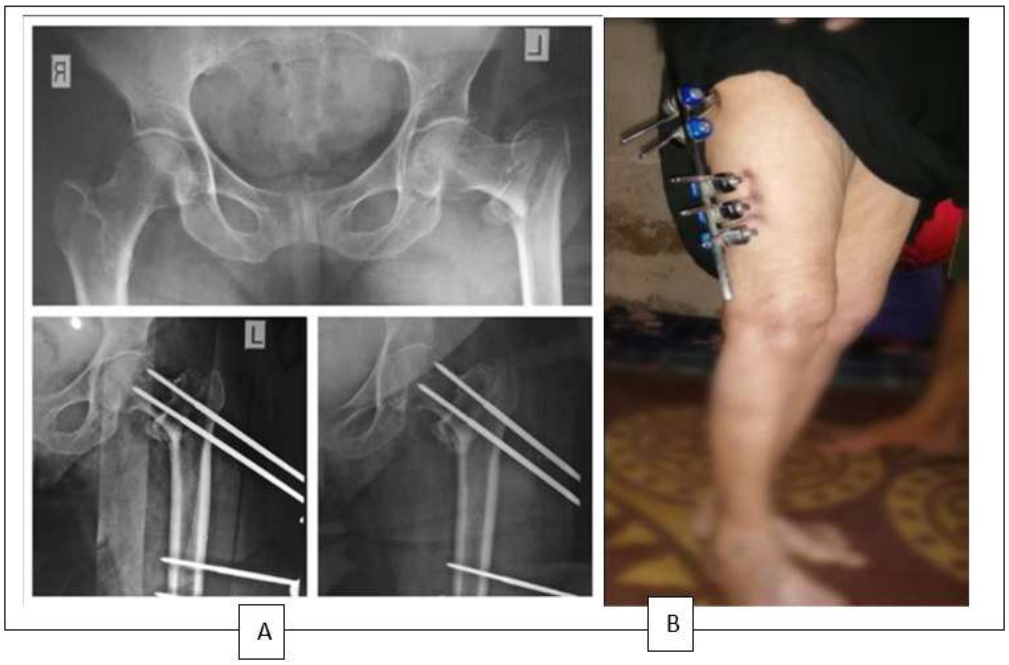

Figure 4. After surgery, A. series of x-rays. B. a patient who is standing and walking with external fixation

Discussion

One of the most common fractures among the elderly are trochanteric hip fractures, and they are a primary cause of fracture-related death and disability [13]. These fractures have a high mortality rate (up to 50%) when treated conservatively.

Except in severely sick or non-ambulatory patients, surgical management is required up to 60%) [14,15].

Geriatric individuals with accompanying medical conditions are high-risk patients for surgery, and every effort should be made to shorten operating time, reduce hospital stay, and allow early mobilization of these patients.

We prospectively follow up on our experience utilizing external fixation to treat elderly patients with trochanteric fractures. We discovered that majority of our patients' functional outcomes at one year were good to excellent, with 80 percent returning to pre-fracture functional levels, as shown in prior trials [16-20].

The modified Hamilton Russell traction is the most effective conservative therapy approach. It necessitates the patient's hospitalization for at least 2 to 3 months, and problems are possible. This is not feasible in most developing countries since it requires a high hospital bed occupancy rate. External fixation may thus be an appropriate therapeutic option for patients who are at a high risk of surgical complications.

Although open reduction and internal fixation of trochanteric fractures is the standard method, there is a high risk of anesthesia or postoperative problems in patients at risk, such as those with ischemic heart disease, chronic pulmonary disease, diabetes mellitus, or severe anemia. The use of a sliding hip screw for intertrochanteric fracture stabilization has been linked to a 4% to 12% loss of fixation rate [21,22].

External fixation also has the advantage of being able to be applied under local anesthesia for patients with poor general health [23]. There were no intraoperative problems, as described in prior investigations [24,25].

In line with prior research, the average intraoperative time for applying the fixator was short (50 minutes) when compared to alternative internal fixation surgical procedures [23,26].

Blood transfusions were not required because blood loss during surgery was minimal compared to other surgical procedures [23,26]. External fixation gear is also less expensive than other internal fixation devices and is readily available in all hospitals.

Due to osteoporosis, gradual weight bearing was postponed for 6-12 weeks after surgery to avoid fracture displacement, implant failure, collapse, Varus malalignment, and femoral head penetration. This differs from what was reported by Refaat et al. [27]. In stable fractures, partial weight bearing was started as soon as the patient was able, while in unstable fractures, it was started after 6 weeks.

Union took an average of 12 weeks, ranging from 10 to 16 weeks. This is consistent with the findings of numerous writers, such as Subasi et al. [19] and Catagni et al. [26] who reported union at 10-18 and 10-12 weeks, respectively.

When compared to gold standard internal fixation techniques that may require general anesthesia, the ability to immediately place an external fixator using local anesthetic and remove it in an outpatient clinic offers it a worthy alternative in elderly, high-risk patients with trochanteric fractures [16].

A common consequence has been documented to be pin-track infection [18]. When hydroxyapatite coated pins are used instead of normal pins, the incidence of pin-track infection is reduced [16].

Regular saline washes, antiseptic dressings, oral antibiotics, and finally the removal of pins and frame following full fracture union were used to treat 18 patients (72%). Pin-track infection occurred in 15 of their 50 patients (30%) treated with the Orthofix external fixator using normal pins, according to Vossinakis and Badras [16] Pin-track problems were found in 45 percent and 60 percent of people in previous studies [11].

The majority of the high-risk, elderly patients in our study had poor bone quality. Varus angulation of an average of 10 degrees was observed in 8 of the 25 instances (32%), which is similar to prior findings [20]. External fixation improves fracture stability in stable trochanteric fractures by increasing callus formation, and it improves load sharing in unstable trochanteric fractures by promoting tension band effect [21].

In conclusion, minimal surgical trauma, low cost, short operative time, minimal anesthetic complications, preservation of fracture hematoma, negligible blood loss, and possibility of application under local anesthesia, frame adjustment, short hospital stay, and removal are all advantages of using external fixator in intertrochanteric fracture in elderly high-risk patients.

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Author contribution

Dr. Alsudany: Manager of Ibnsina Orthopedic Training Center, surgeon, study designer and writing manuscript. Dr. Alhamashi: Surgeon and data collector. Dr. Al-Abbody: Surgeon and data collector. Dr. Ismael: Acquiring and analyzing data.

Conflict of interest

There are none for the authors to declare.

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Klebsiella pneumoniae in Hospital Acquired and Community Acquired Urinary Tract Infections in an Iraqi Cohort: Frequency, Antibiotic Susceptibility and the Percentage of bla KPC Resistance Gene

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Abstract

Background *Klebsiella pneumoniae* (*K. pneumoniae*) is an important pathogen in hospital and community-acquired urinary tract infections (HAUTI and CAUTI). It is showing a high resistance towards many antibiotics

Objective This study aims to estimate the percentage of *K. pneumoniae* in HAUTIs and CAUTIs, antibiotic resistance, extended spectrum bata lactamase (ESBL), and detect the beta lactamase *K. pneumoniae* carbapenemases (bla KPC) resistance gene and presence of mutation in this gene.

Methods A total of 200 urine samples were collected randomly; *K. pneumoniae* was cultured and isolated on MacConkey agar, antibiotic sensitivity testing and ESBL detection were performed by Vitek 2 system, then DNA was extracted, polymerase chain reaction and sequencing for KPC gene were done.

Results There was 7% (14 isolates) of *K. pneumoniae* (50%) HAUTI and (50%) CAUTI. ESBL was positive in 3 isolates (21.43%). Percentage of positive bla KPC gene was 42.8%. KPC gene sequencing showed high rate of mutation in positive isolates reaching to 50 different nucleotides.

Conclusion This work has increased the knowledge on *K. pneumoniae* causing HAUTI and CAUTI in Iraqi patients.

Keywords *Klebsiella pneumoniae*, hospital acquired urinary tract infection, community acquired urinary tract infection, ESBL, bla KPC gene.

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List of abbreviations: , ANOVA = Analysis of variance, bla KPC gene = beta lactamase *Klebsiella pneumoniae* carbapenemase gene, CAUTI = Community acquired urinary tract infection, CLSI = Clinical and laboratory standard institute, DDT = Disc diffusion test, ESBL = Extended spectrum beta lactamase, *K. pneumoniae* = *Klebsiella pneumoniae*, HAUTI = Hospital acquired urinary tract infection, KPC = *Klebsiella pneumoniae* carbapenemases, MSAV = Multiple sequence alignment viewer, OD = Optical density, WHO = World Health Organization

Introduction

Klebsiella pneumoniae (*K. pneumoniae*) is a significant pathogen in nosocomial infections [(1)], also it is the second most common etiological agent involved in community-acquired urinary tract infections (CAUTI) [(2)]. Nosocomial health-care-associated infection is described as an infection rising in a patient hospitalized for more than 48 hours

before the onset of signs and symptoms consistent with the infection [3]. Non-nosocomial health-care-associated infection is explained as an infection detected within 48 hours of admission in an outpatient with extended healthcare contact [4].

K. pneumoniae is showing a high resistance to a broad spectrum of antibiotics including beta-lactam antibiotics, fluoroquinolones, and aminoglycosides [5,6]. This resistance is leading to arising worldwide problem with the choice of the proper antibiotic treatment for hospital-acquired infections [7].

KPC gene (Klebsiella pneumoniae carbapenemase) belongs to the Ambler class A, Bush subgroup 2f, serine based carbapenemases, which are active against all beta-lactams, including the carbapenems [8].

This study aimed to estimate the percentage of *K. pneumoniae* in HAUTI and CAUTI, study antibiotic resistance and extended spectrum beta lactamase (ESBL), detect the resistance gene (bla KPC) and determine the presence of mutation in bla KPC resistance gene.

Methods

Subjects

A total of (200) urine samples (100 from HAUTI and 100 CAUTI) from (one day to 80 years old) patients with UTI were collected randomly from the hospital laboratory of Al-Imamein Al-Kadhimein Medical City Baghdad, Iraq. The UTI patients were diagnosed as HAUTI or CAUTI by the specialists in a period from October 2020 to February 2021. This study was approved by the by the Institutional Review Board of the College of Medicine, Al-Nahrain University (No.1451) and conducted in the Microbiology Department of this College. All collected urine samples were cultured on MacConkey Agar medium (Biolab, Hungary), incubated at 37°C for 24 hours in aerobic conditions. The isolated bacteria were recognized according to morphological characteristics and the identification of the grown colonies was confirmed by the VITEK-2 system.

Antimicrobial susceptibility tests

Resistance patterns of Klebsiella isolates to 14 different antibiotics were detected by disk diffusion test (DDT) and by VITEK 2 System according to Clinical and Laboratory Standards Institutes (CLSI) 2016 [9].

Detection of ESBL by double disk diffusion method

A disk containing amoxicillin/clavulanate (AMC) is placed in proximity to a disk containing ceftazidime (CAZ) or another oxyimino-cephalosporin. The clavulanate in the amoxicillin-clavulanate disk diffuses through the agar and inhibits the lactamase surrounding the ceftazidime disk. Enhancement of the zone of the ceftazidime disk on the side facing the amoxicillin/clavulanate disk is interpreted as a positive test [10].

Molecular study

DNA Extraction

DNA was extracted according to the manufacture instructions by using Gram negative bacteria Wizard genomic DNA purification Kit. DNA concentration and purity was determined by Nano-drop system.

Polymerase chain reaction (PCR) screening bla KPC resistance gene

Approximately 1.5 µl from both forward and reverse primers sequence (Table 1) were apply to yield a DNA fragment of (340) bp using conventional PCR in a total volume 20 µl of reaction mixture. Thermal profile of reaction was showed in table (2).

Agarose gel electrophoresis for detection of PCR products

Five µl of each PCR product and negative control was subjected into 1.5% (wt/vol) agarose gel electrophoresis with ethidium bromide (0.5 µg /ml; Promega, USA) at 7 V/cm for 1.5 hr. Five microliters of the 100bp DNA ladder was included as a marker during PCR products electrophoresis. Amplicon visualization was performed using an UV light

trans illuminator and then photographed by mobile device camera (iPhone) [13].

Table 1. Oligonucleotide's primers and product size of bla KPC resistance gene

Gene		Nucleotide sequences (5' ⟶ 3')	Products *bp*	Reference
***bla*KPC**	**F**	TGTTGCTGAAGGAGTTGGGC	**340**	[11]
	R	ACGACGGCATAGTCATTTGC		

Table 2. Polymerase chain reaction program for amplification of bla KPC resistance gene by thermal cycler

No.	Steps	Temperature	Time	No. of cycles
1	Initial denaturation	95˚C	5 minutes	1
2	Denaturation	95˚C	1 minute	
3	Annealing	56˚C	1 minute	35
4	Extension	72˚C	1 minutes	
5	Final extension	72˚C	5 minutes	1

PCR product of bla KPC resistance gene

In the conventional PCR were sequenced in forward direction using the same primers used in the PCR reactions; according to the Macrogen, Inc. protocol (Korea) Results were compared according to data base of Gene bank.

Statistical methods

The statistical analysis system program includes Fisher's exact test that used to analyze data of this study. Entry of data into Excel systems and the exact tests were achieved by statistical package for social sciences (SPSS) version 20 (2020).

Results

The percentage of *K. pneumoniae* isolates was 7% (14 isolates out of 200 urine samples). Regarding gender distribution, the percentage of females was 42.9% (6 isolates) and males were 57.1% (8 isolates). In the current study, the percentage of hospital acquired urinary tract infection patients were 50% and community acquired urinary tract infection patients were 50%.

Antibiotic resistance of *K. pneumoniae* isolates

The results of the antimicrobial susceptibility test showed that all *K. pneumoniae* isolated from HAUTI and CAUTI patients were complete resistant (100%) to Amoxicillin/clavulanic acid; while there was a high rate of resistance (71.4%) to Aztreonam, trimethoprim/sulfamethoxazole and Trimethoprim in HAUTI and CAUTI, also resistance to Cefotaxime and Ceftazidime were (57.1% and 71.4%), whereas Ceftriaxone were (42.8% and 71.4%) in both HAUTI and CAUTI. The resistance was moderate to Ciprofloxacin (57.1%), Gentamicin (57.1% and 28.5%), Norfloxacin (42.8%) for both, Cefoxitin and Amikacin (42.8% and 14.2%), Meropenem (28.5% and 42.8%) and low to Imipenem (28.5% and 28.5%) in HAUTI and CAUTI respectively. Antibiotic resistance to ceftriaxone, meropenem, ceftazidime and cefotaxime is higher in CAUTI, while antibiotic resistance to cefoxitin, gentamicin and amikacin is higher in HAUTI as shown in the table (3).

Table 3. The percentages of resistance of *Klebsiella pneumoniae* isolates to different antibiotics by disc-diffusion method in HAUTI and CAUTI

	ATM	CRO	NOR	CTX	FOX	CIP	AMC	SXT	CN	AK	IPM	MEM	CAZ	TMP
% of resistance in HAUTI	71.4	42.8	42.8	57.1	42.8	57.1	100	71.4	57.1	42.8	28.5	28.5	57.1	71.4
% of resistance in CAUTI	71.4	71.4	42.8	71.4	14.2	57.1	100	71.4	28.5	14.2	28.5	42.8	71.4	71.4

Aztreonam (ATM), Ceftriaxone (CRO), Norfloxacin (NOR), Cefotaxime (CTX), Cefoxitin (FOX), Ciprofloxacin (CIP), Amoxicillin/Clavulanic acid (AMC), Trimethoprim/sulfamethoxazole (SXT), Gentamicin (CN), Amikacin (AK), Imipenem (IPM), Meropenem (MEM), Ceftazidime (CAZ), Trimethoprim (TMP)

Detection of Extended Spectrum Beta Lactamase (ESBL)

The percentage of positive ESBL in this study was 21.43% (3 isolates). The percentage of ESBL was (28.5%) in CAUTI (2 isolates) and (14.28%) in HAUTI (1 isolate). The ESBL positive *K. pneumoniae* isolates, were resistant to all 14 antibiotics used in this study except one isolate that was resistant to ciprofloxacin, Norfloxacin, Amoxicillin/Clavulanic acid only.

Polymerase chain reaction screening for bla KPC resistance gene

Sequence amplification of Klebsiella pneumoniae bla KPC resistance gene was done by PCR technique with product size 340 bp as shown in figure (1). Current study revealed that percentage of bla KPC gene was 42.8% (6 isolates).

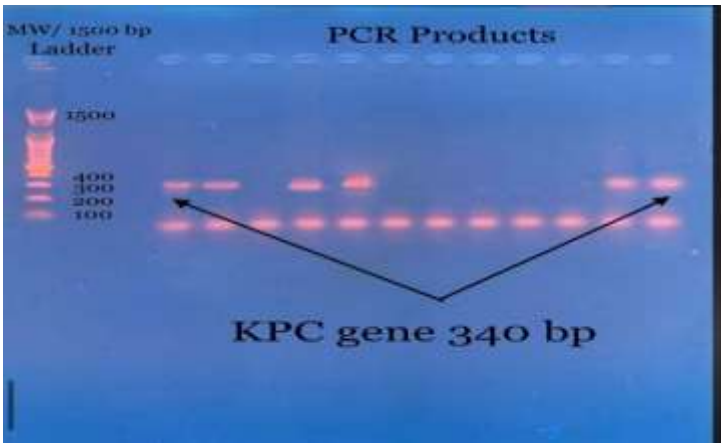

Figure 1. Gel electrophoresis of PCR products (340 bp) for bla KPC resistance gene. Lane 1: 100bp ladder. Lanes 2-13: PCR products of Klebsiella pneumoniae isolates. (1.5% agarose, 7 v/cm, 45 min)

Study KPC resistance gene

The results of antimicrobial susceptibility test showed that all Klebsiella pneumoniae isolates were completely resistant (100%) to Amoxicillin/clavulanic acid whether KPC gene positive or negative; KPC negative isolates showed a higher rate of resistance to Aztreonam (87.5%), Ceftriaxone (62.5%), Norfloxacin (50%), Cefotaxime (75%), Ciprofloxacin (62.5%), Trimethoprim/sulfamethoxazole (75%), Imipenem (37.5%), Meropenem (37.5%), and Trimethoprim (75%). While KPC positive isolates showed a slightly higher resistance for Cefoxitin (33.3%), Gentamicin (50%), Amikacin (33.3%), and Ceftazidime (66.6%) as shown in table (4).

Association Between ESBL and KPC gene

In current study there is no significant association between ESBL and KPC gene as shown in table (5).

Table 4. Association between percentages of resistance bla KPC gene negative and bla KPC gene positive with different antibiotics

	ATM	CRO	NOR	CTX	FOX	CIP	AMC	SXT	CN	AK	IPM	MEM	CAZ	TMP
% of resistance in KPC gene negative	87.5	62.5	50	75	25	62.5	100	75	37.5	25	37.5	37.5	62.5	75
% of resistance in KPC gene positive	66.6	50	33.3	50	33.3	50	100	66.6	50	33.3	16.6	33.3	66.6	66.6

Aztreonam (ATM), Ceftriaxone (CRO), Norfloxacin (NOR), Cefotaxime (CTX), Cefoxitin (FOX), Ciprofloxacin (CIP), Amoxicillin/Clavulanic acid (AMC), Trimethoprim/sulfamethoxazole (SXT), Gentamicin (CN), Amikacin (AK), Imipenem (IPM), Meropenem (MEM), Ceftazidime (CAZ), Trimethoprim (TMP)

Table 5. Association between ESBL and KPC gene

ESBL		KPC_gene		Total
		Absent	Present	
Negative	Count	6	5	11
	% within ESBL	54.5%	45.5%	100.0%
	% within KPC gene	75.0%	83.3%	78.6%
Positive	Count	2	1	3
	% within ESBL	66.7%	33.3%	100.0%
	% within KPC gene	25.0%	16.7%	21.4%
Total	Count	8	6	14
	% within ESBL	57.1%	42.9%	100.0%
	% within KPC gene	100.0%	100.0%	100.0%

KPC resistance gene sequencing

Gene sequencing is backed by automated DNA sequencing methods and computer software (BLAST which means Basic Local Alignment Search Tool) to assemble the enormous sequence data.

This gene is highly diveresed. Despite it is chromosomal gene; but exposed to highly mutation. So it might affect antibiogram and selective pressure of mutated strain. Z1, Z2, Z3, Z4 and Z5 are blasting with gene bank. The closest strain to Z1 is E16KP0102 which has been submitted to Gene bank with 98% identities and 1% Gaps. The types of mutation are insertion and transition as seen in figure (2).

The closest strain to Z2 is KPN1344 which has been submitted to Gene bank with 94% identities and 2% Gaps. The types of mutation are insertion, transition and transversion as seen in figure (3).

The closest strain to Z3 is E17KP0052 which has been submitted to Gene bank with 86% identities and 3% Gaps. The types of mutation are insertion, transition and transversion as shown in figure (4).

The closest strain to Z4 is KPN236 which has been submitted to Gene bank with 99% identities and 0% Gaps. The type of mutation is insertion as shown in figure (5).

The closest strain to Z5 is E17KP0052 which has been submitted to Gene bank with 98% identities and 1% Gaps. The types of mutation are insertion and transversion as shown in figure (6).

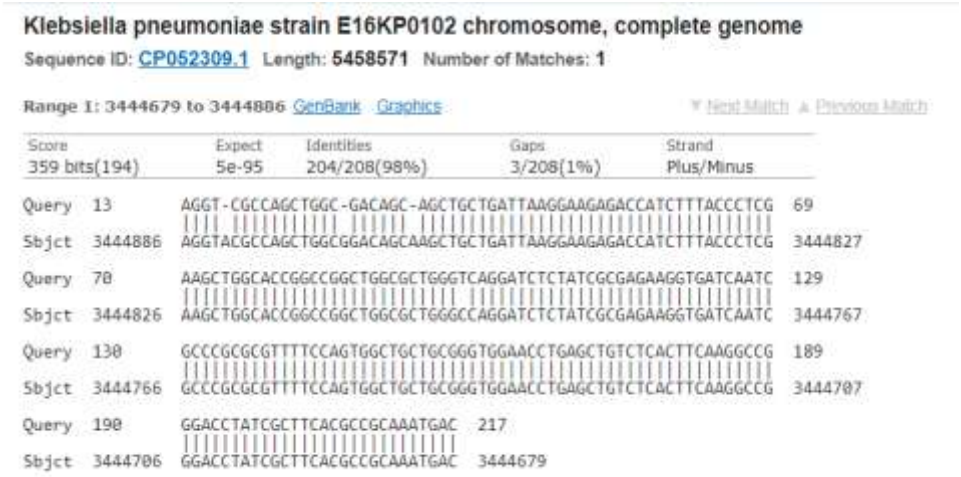

Figure 2. Gene blasting of Z1: Closest strain in gene bank is E16KP0102 within 98% Identities. Query means the studied strain, while subject means strain in the gene bank

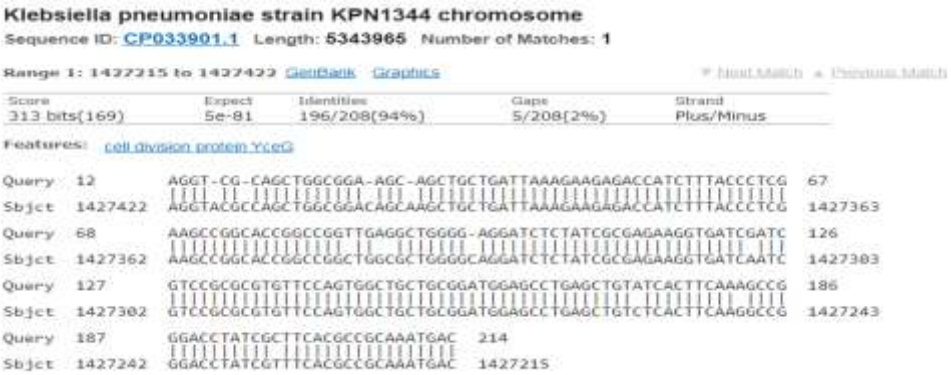

Figure 3. Gene blasting of Z2: Closest strain in gene bank is KPN1344 within 94% Identities. Query means the studied strain, while subject means strain in the gene bank

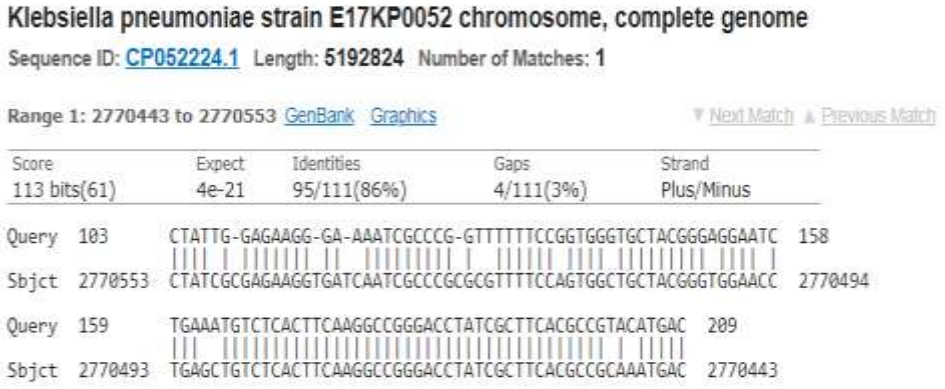

Figure 4. Gene blasting of Z3: Closest strain in gene bank is E17KP0052 within 86% Identities. Query means the studied strain, while Subject means strain in the gene bank

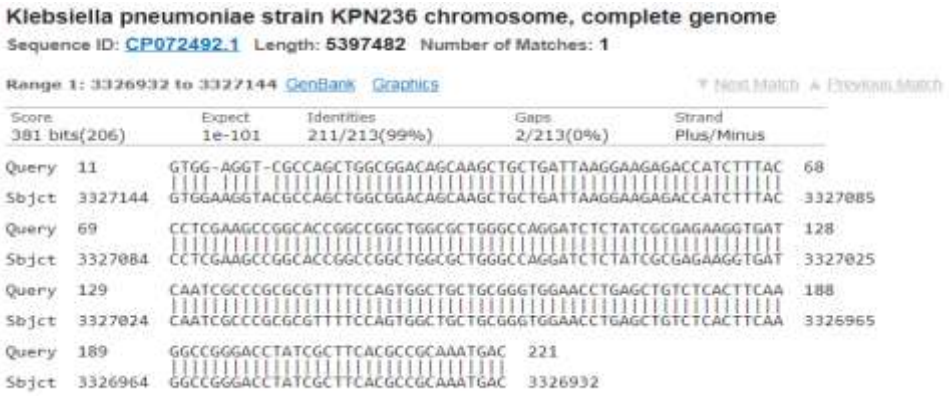

Figure 5. Gene blasting of Z4: Closest strain in gene bank is KPN236 within 99% Identities. Query means the studied strain, while Subject means strain in the gene bank

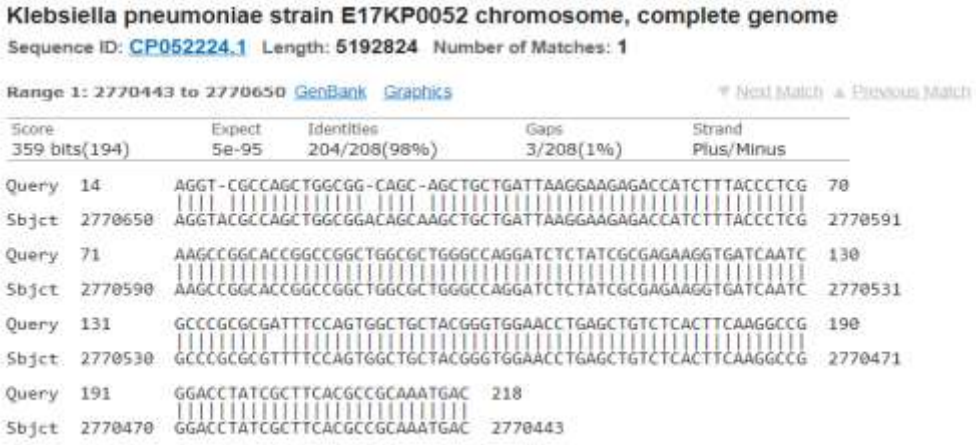

Figure 6. Gene blasting of Z5: Closest strain in gene bank is E17KP0052 within 98% Identities. Query means the studied strain, while Subject means strain in the gene bank

Discussion

In the present study the percentage of *K. pneumoniae* in HAUTI and CAUTI were 50% and 50% respectively. In a study published in Moroco by El Bouamri et al. in 2015 [(14)], the prevalence of *K. pneumoniae* causing UTI was (22%) (321 isolates), in which (82%) (263 isolates) of isolated strains of *K. pneumoniae* caused CAUTIs. Whereas in study conducted in Portugal by Caneiras et al. in 2019 [(15)], the frequency of total *K. pneumoniae* isolates was 81 (50 CAUTI and 31 HAUTI). This difference in the prevalence *of K. pneumoniae* in the current study and previous studies could be due to the larger sample size included in mentioned studies, differences in geographical areas, in addition to the differences of methods used in each study and the skill of researchers.

Emergence of antibiotic resistance is an important process due to its capacity to resist and acquire various resistance mechanisms against antibacterial drugs.

The results of this study showed that 100% of *K. pneumoniae* isolates were resistant to Amoxicillin/clavulanic acid in both HAUTI and CAUTI. Similar findings in a study conducted in Iran by Ranjbar et al. in 2020 [(16)], who reported that, the high rate of resistance to Amoxicillin/clavulanic acid was (95.65%) making this antibiotic a poor choice for

treatment of infections with Klebsiella pneumoniae that causes hospital acquired urinary tract infection.

The current study included high rate of resistance (71.4%) to Aztreonam, trimethoprim/sulfamethoxazole and Trimethoprim in HAUTI and CAUTI. In a study conducted in Iran by Ranjbar et al. in 2020 [16], that percentage of resistance to Aztreonam was (74.78%,) which is compatible with the current results. In study that done in South Africa by Vasaikar et al. in 2017 [17], showed high percentage of resistance to Trimethoprim/Sulfamethoxazole (70.8%) and aztreonam (62.4%), which agree with the current one.

In the present study resistance to Cefotaxime and Ceftazidime were (57.1% and 71.4%), whereas Ceftriaxone was (42.8% and 71.4%), Ciprofloxacin (57.1% and 57.1%) in HAUTI and CAUTI respectively. This study is in compatible with the study done in north-central Iran by Yazdansetad et al. in 2019 [18], who reported that the percentage of resistance of Cefotaxime (40%), Ceftazidime (55%) and Ciprofloxacin (60%) in HAUTI.

The study conducted in Bobo-Dioulasso by Sagna et al. in 2019 [19], found the resistance to Cefotaxime was (56.4%), Ceftazidime (49.1%), Ceftriaxone (1.8%), which disagree with the current results.

In the present study the resistance was moderate – to - low to Gentamicin (57.1% and 28.5%), Norfloxacin (42.8% and 42.8%), Cefoxitin and Amikacin (42.8% and 14.2%), Meropenem (28.5% and 42.8%) and Imipenem (28.5% and 28.5%) in HAUTI and CAUTI respectively. The current study shows a kind of compatibility with a study published in South Africa by Vasaikar et al. in 2017 [18], the resistance to Amikacin (5%), Imipenem (0%), Meropenem (0%), Norfloxacin (1.5%), Cefoxitin (8.9%), Ciprofloxacin (29.7%) were low. Also, this study agrees with the study published in north-central Iran by Yazdansetad et al. in 2019 [18], the resistance of Gentamicin was (30%), Norfloxacin (65%), Amikacin (50%) and Imipenem (15%) in nosocomial UTI.

In the current study, the percentage of the positive ESBL was 21.43%. (28.5% positive in CAUTI and 14.28% positive in HAUTI). While in a study that published in Kurnool by Sarojamma et al. in 2011 [20], ESBL was positive in (28%) of the hospital isolates compared to (6%) of community isolates.

Antibiotic resistance has been increased in Iraq because these antibiotics are taken without prescription (abuse of antibiotics). Also, the bad mark that imported, bad storage of antibiotics leads to develop MDR (multidrug resistance) and XDR (extensively drug resistance) strains.

The current study exhibited a percentage of positive ESBL as 21.43%. (28.5% in CAUTI and 14.28% in HAUTI). Prevalence of ESBL producers in any hospital depends upon various factors like antibiotic policy, the carriage rate among the hospital personnel, and the type of disinfection used especially in ICU [21]. In a study conducted in in Africa by Sirot et al. in 1987 [22], the percentage of positive ESBL was 22.8% in which is compatible with the current results. In a study done in China by 23. Rodríguez-Martínez et al. in 2011 [23], positive ESBL was 49.2% which is not compatible with the present results. This differences in prevalence of high resistance rate of antimicrobials and high prevalence of ESBL producing *K. pneumoniae* strains may be attributable to the differences degrees in virulence strains, antimicrobial stewardship program, geographic differences and infection control practices [24,25].

In this study, bla KPC resistance gene was identified in *K. pneumoniae* isolates using PCR technique. Current study revealed that the percentage of bla KPC gene by conventional PCR was 42.8%. Whereas in study that conducted in Brazil by Ferreira et al. in 2018 [26], all isolates (100%) were positive for the bla KPC gene which is not compatible with the current results.

The results of antimicrobial susceptibility test showed that all Klebsiella pneumoniae isolates were complete resistant (100%) to Amoxicillin/clavulanic acid regardless of whether KPC gene positive or KPC gene negative; while there is high rate of resistance (87.5%) to Aztreonam, (75%) to each cefotaxime, Trimethoprim/Sulfamethoxazole

and Trimethoprim alone in KPC gene negative. While resistance to these mentioned antibiotics were moderate in KPC gene positive. Also, the resistance was moderate - to- low to Ceftazidime (62.5 % and 66.6 %), Ceftriaxone and Ciprofloxacin (62.5 % and 50 %), Gentamicin (37.5 % and 50 %), Norfloxacin (50 % and 33.3 %), Cefoxitin and Amikacin (25 % and 33.3 %), Meropenem (37.5 % and 33.3 %) and Imipenem (37.5 % and 16.6 %) within each KPC gene negative and KPC gene positive respectively.

In study conducted in Iran by Bina et al. in 2015 [27], 14.65% of the *K. pneumoniae* strains were resistant to carbapenems. The antibiotic susceptibility test results exhibited that the highest resistance to the antibiotic were related to Gentamicin and Cefepime [28], whereas, the lowest rate was related to Imipenem, Meropenem, Ertapenem and Ceftazidime [29]. In the MHT (modified Hodge test), 80.5% strains were positive for KPC. All 41 carbapenem resistant *K. pneumoniae* isolates were negative for bla-KPC gene.

While, other studies in the USA [30], China [31] and Italy [32] confirm the presence of the bla-KPC gene in carbapenem resistant *K. pneumoniae* isolates by PCR. This difference can be due to decreased susceptibility to at least one extended-spectrum Cephalosporin [33] and another mechanism such as of carbapenem resistance as a result of a combination of an ESBL or AmpC-type enzyme with porin loss [34,35].

In the current study, there was no significant association between ESBL and bla KPC gene. While in a study done in Brazil by Peirano et al. in2019 [36], the isolates that were positive for KPC were ESBL phenotype production. ESBL-encoding gene bla KPC-2 (six isolates) carbapenemase was detected among isolates. Most KPC producers are ESBL producers as well which is not compatible with the current results [37].

The present study observed that KPC gene is highly diverse. Despite it is a chromosomal gene; it is highly mutating. This might affect antibiogram and selective pressure of mutated strain. As Study that was published in Virginia by Sheppard et al. in 2016 [38], explained that KPC leads to multidrug resistance, with KPC-producing Enterobacteriaceae becoming increasingly widespread in nosocomial infections. This gene revealing a high level of genetic diversity, with the most prevalent lineage being ST941 [39].

The power of the current study was finding Imipenem as the most effective antibiotic can be used for treatment of UTI caused by *K. pneumoniae*. Also, finding different types of mutation of bla KPC gene which have positive effect in antibiotic susceptibility of *K. pneumoniae* isolates.

While the limitations in the present study was as in most empirical studies, it was limited by the time, Sample size, location of study, sampling technique used and variables selected.

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Author contribution

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Conflict of interest

There is no conflict of interest.

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Association of Anti-Coxsackie Virus-B IgG with Autoantibodies Related to Type 1 Diabetes Mellitus

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Abstract

Background Coxsackievirus B is a virus may cause type 1 diabetes. There are links between Coxsackievirus B infections and type 1 diabetes. The presence of autoantibodies in pancreatic beta cells has been linked to the development of type 1 diabetes following Coxsackievirus B infection.

Objective To detect autoantibodies in patients with type 1 diabetes. Also, to find if there is an association between pancreatic beta cell autoantibodies and Coxsackievirus-B IgG.

Methods This study was done from January to March 2021, it included two groups of 75 children; their ages ranged from one month to fifteen years. Children with type 1 diabetes were admitted to the diabetic and endocrine glands center in Thi-Qar governorate, whereas children without diabetes (control group) were admitted to Bint Al-Huda Children's Hospital in Nasiriyah/Thi-Qar. Venous blood was taken from each person for estimation of random blood sugar, serum fructosamine, and HbA1c in the laboratory of the diabetic and endocrine glands center. Pancreatic beta cell autoantibodies and anti-Coxsackievirus-B IgG was detected by enzyme-linked immunosorbent assay. The statistical analyses were done using SPSS 25. The study population P-values below 0.05 to be statistically significant. The study was authorized by Al-Nahrain University's Institutional Review Board and parental consent was taken.

Results The patients had significantly greater levels of random blood sugar, fructosamine, and HbA1c than healthy controls. Anti-islet antigen, anti-islet cell, anti-glutamic acid decarboxylase, and anti-Coxsackievirus-B IgG antibody titers were greater in patients than controls. The majority of autoantibodies tested correlated with Coxsackievirus-B IgG antibodies.

Conclusion Anti-Coxsackievirus-B IgG antibody positivity was associated with autoantibodies related to type 1 diabetes mellitus.

Keywords Coxsackievirus B, type 1 diabetes, autoantibodies

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List of abbreviations: CVB = Coxsackievirus B, DCM = Dilated cardiomyopathy, ELISA = Enzyme-linked immunosorbent test, FBS = Fasting blood sugar, GADA = Glutamic acid decarboxylase 65 autoantibodies, HbA1c=hemoglobin A1c, HEVs = Human enteroviruses, HFMD = Hand-foot-and-mouth disease, IA-2A = Tyrosine phosphatase–like insulinoma antigen-2 antibodies, IAA = Insulin autoantibodies, T1DM = Type 1 diabetes mellitus, ZnT8 = Zinc transporter 8 autoantibodies

Introduction

Coxsackievirus B (CVB) is classified as an enterovirus of the Picornaviridae family. CVB is a single-stranded RNA virus that infects humans [(1)]. CVB viruses are classified into six serotypes, designated as CVB1–6, and in the majority of cases, infections with these viruses are asymptomatic or result in mild symptoms that are similar to those of the

common cold or flu [2]. CVB, on the other hand is linked to several of potentially life-threatening disorders, including encephalitis and aseptic meningitis [1], myocarditis and chronic dilated cardiomyopathy (DCM) [3], pancreatitis [2], and hand, foot, and mouth disease (HFMD) [4]. There are also substantial connections between CVB infections and the chronic autoimmune illness type one diabetes (T1DM), showing that these viruses are a significant contributor to an expanding range of diseases with significant clinical and economic consequences [5].

Specific CVB serotypes are shown to be related to each of the disorders listed above. As an example, CVB3 infections are linked to viral myocarditis and DCM [6], CVB5 infections are linked to encephalitis and aseptic meningitis outbreaks [7], and CVB1 is linked to the induction of β-cell autoimmunity, which is linked to T1DM [8]. In type one diabetes, beta cells are destroyed by an antibody that affects the pancreas, leading to the loss of insulin-producing beta cells [9].

Diagnosing T1DM from other forms is particularly important in its treatment because it helps with medication selection, evaluation of illness prognosis, and determining the risk of diabetes development in family members of the diabetic patient. T1DM is distinguished from other forms by the presence or absence of insulin production [8].

A large number of autoantibodies have been discovered in pancreatic beta cells, and it has been postulated that these autoantibodies may act significant roles at the beginning of autoimmune islet destruction [7]. The autoantibodies that have developed as a result of islet destruction are most likely directed towards insulin itself as their principal target. Glutamic acid decarboxylase 65 autoantibodies (GADA), insulin autoantibodies (IAA), and zinc transporter 8 autoantibodies (ZnT8) may be utilized to diagnose T1DM [7].

People who have just been diagnosed with T1DM are nearly always found to have one or more autoantibodies at the time of their diagnosis, which is virtually always accurate [4]. The prevalence of type one diabetes in newly diagnosed individuals is increasing. The presence of islet cell autoantibodies (ICA) is discovered in 85 percent of cases [5], the presence of GADA is detected in 70%, and the presence of Tyrosine phosphatase–like insulinoma antigen-2 antibodies (IA-2A) is detected in 58% of patients [6].

The objectives of this study were to identify people who have autoantibodies associated with T1DM and to determine whether or not there is a correlation between autoantibodies against pancreatic beta cells and IgG against coxsackievirus-B.

Methods

Blood sample was taken from a total of 75 patients (38 males + 37 females), that were recently diagnosed with T1DM at the Center of Diabetic and Endocrine Glands in Thi-Qar Governorate throughout three-month period from January to March 2021. In addition, 75 control blood samples (31 males + 44 females), were collected from children visiting Bint Al-Huda Children's Hospital. Blood samples were collected from non-diabetic children who seemed healthy and had no family history of T1DM (left over). This group of people included boys and females who were in the same age range as the patient population (ranging from one month to 15 years).

Using a disposable syringe, venous blood was taken, providing a volume of 5 ml. Each blood sample was separated into two parts using a sterile plane tube. In part one, 3 ml of blood were drawn and left to coagulate at room temperature in order to separate the serum from the remainder of the sample.

The enzyme-linked immunosorbent assay (ELISA) was used to evaluate anti-CoxV-B IgG levels and autoimmune antibodies (by using kits manufactured in Shenzhen New Industries/China) in serum that had been stored at -20°C. The quantitative measurement of anti-CoxV-B IgG was developed using the cut off value as a reference. In order to determine HbA1c levels, the remaining 2 ml of blood were

drawn and immediately deposited into an Ethylene Diamine Tetra Acetic Acid (EDTA) tube for further processing.

Statistics were carried out with the aid of the statistical package for the social sciences (SPSS) software version 25. The mean and standard deviation of data with a normal distribution, as well as the T test, were calculated. If data having a non-normal distribution were significant, the Mann Whitney U test was used to examine the median and range of each variable. When used in conjunction with a receiver operating characteristic curve (ROC), anti-CoxV-B IgG has been shown to be diagnostically beneficial in the discrimination between patients and controls. It was determined that a statistically significant difference existed when the P-value was less than 0.05, according to the findings of the research.

Results

Biochemical tests related to T1DM

Fasting blood sugar (FBS) and HbA1c were significantly greater in patients (268.92±86.21 mg/dl and 8.23±1.76%, respectively) than in controls (113.94±17.08 mg/dl and 4.76±0.5%, respectively) (P<0.001). Also, patients had greater levels of fructosamine than controls (5.42±1.0 mmol/l versus 1.91±0.18 mmol/l), (Table 1).

Table 1. Biochemical tests related to T1DM

Tests		Patients (n=75)	Control (n=75)	p value☐
FBS (mg/dl)	Mean±SD Range	268.92±86.21 135-560	113.94±17.08 84-158	< 0.001
Fructosamine (mmol/l)	Mean±SD Range	5.42±1.0 3.8-7.9	1.91±0.18 1.7-2.5	< 0.001
HbA1c, %	Mean±SD Range	8.23±1.76 6.7-12.7	4.76±0.5 4.2-6.1	< 0.001

☐Student t-test

Autoantibodies tests related to T1DM

According to table (2), the median levels of anti-Islet antigen antibody (Anti-IA2), islet cell antibody (ICA), and glutamic acid decarboxylase antibodies (GAD65) in patients were 34.8 U/ml, 32.7 U/ml, and 43.8 IU/ml, respectively, compared to 16.9 U/ml, 17.3 U/ml, and 14.8 IU/ml in controls with highly significant differences.

Detection of anti-CoxV-B IgG antibody

Anti-CoxV-B IgG antibodies titer in patients was 0.85 pg/ml (range 0.25-3.3 pg/ml), which was higher than the median serum level in controls (median= 0.71 pg/ml, range0.47-0.97 pg/ml), showing that patients had higher levels of antibodies than controls (p=0.005). Ten patients (13.33%) tested positive for anti-CoxV-B IgG antibodies, while none of the children in the control group tested positive, according to the manufacturer's recommendations of kit (Figure 1).

Table 2. T1DM-Related autoantibodies

Autoantibodies		Patients (n=75)	Control (n=75)	P value☐
Anti-IA2 (U/ml)	Mean±SD	38.2±10.16	16.16±5.45	< 0.001
	Median	34.8	16.9	
	Range	25.9-71.2	6.8-27.3	
ICA (U/ml)	Mean±SD	34.31±6.35	16.27±5.19	< 0.001
	Median	32.7	17.3	
	Range	24.9-59.6	7.9-27.0	
GAD (IU/ml)	Mean±SD	49.56±13.07	15.36±4.84	< 0.001
	Median	43.8	14.8	
	Range	31.6-85.2	7.9-28	

☐Mann Whitney U test

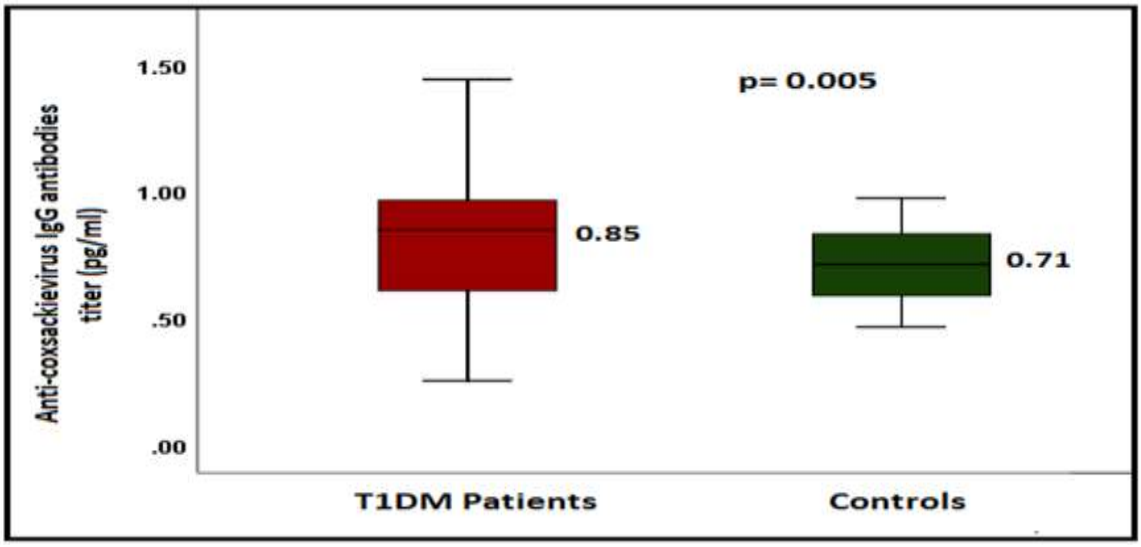

Figure 1. Median level of anti-Coxsackievirus-B IgG antibodies titer in T1DM patients and controls

Association of anti-CoxV-B IgG antibody positivity with autoantibodies tests related to T1DM

Most autoantibodies tests showed significant association with anti-CoxV-B IgG antibody. The median level of anti-IA2, ICA and GAD65 in patients positive for anti-CoxV-B IgG antibodies was 53.15 U/ml, 41.75 U/ml and 58.6 U/ml, respectively compared with 34.6 U/ml, 31.9 U/ml and 43.7 U/ml, respectively, in patients negative for anti-CoxV-B IgG antibodies with highly significant differences (Table 3).

Table 3. Association of anti-Coxsackievirus-B IgG antibodies positivity with autoantibodies tests related to T1DM

Autoantibodies		IgG-positive (n=10)	IgG-negative (n=65)	p- value
Anti-IA2 (U/ml)	Median Range	53.15 87.8-71.2	34.6 25.9-59.8	< 0.001☐
ICA (U/ml)	Median Range	41.75 36.5-59.6	31.9 24.9-41.9	< 0.001☐
GAD (IU/ml)	Median Range	58.6 41.6-74.9	43.7 31.6-85.2	0.013☐

☐Mann Whitney U test

Discussion

In this study, researchers found a statistically significant positive connection between FBS, HbA1c, and fructosamine in persons with T1DM. Like our findings, Zamanfar et al. in Iran (9), 10. Basu et al. in India [10], Belhiba et al. in Morocco [11], have reported positive correlations between FBS, HbA1c, and fructosamine values with diabetic patients.

According to the data, HbAlc has been found to be a strong predictor of fructosamine. On the other hand, FBS and fructosamine have been found to be strong predictors of HbA1c. Fructosamine offers a blood sugar state in 2-3 weeks, but HbA1c provides a blood sugar status in 2-3 months and is inconsistent with red blood cell lifespan (RBCs). Younger people with fewer red blood cells had lower HbA1c levels, whereas older people with more RBC have higher HbA1c values [12].

T1DM had greater levels of anti-IA2, anti-ICA, and GAD65 antibodies than healthy controls. Zamanfar et al. in Iran [9], Basu et al. in India [10], Belhibaet al. in Morocco [11], and Bravis et al. in the United Kingdom [13] all came to a similar result.

Beta cell autoimmunity is defined by the presence of diabetes-associated autoantibodies in the bloodstream, which indicates that the cells are being attacked. It is necessary to differentiate between diabetes types 1, 2, and monogenic diabetes using these indicators, which are crucial diagnostic tools. Among the signs and symptoms of T1DM include the existence of GAD65, anti-IA-2, ICA, and ZnT8 autoantibodies [9].

Despite the fact that most persons with a single autoantibody do not develop T1DM, children with two or more serum autoantibodies have an 84% chance of having the condition by the age of eighteen. Because many autoantibodies enhance the risk of progression, the stages of T1DM have been reclassified and reinterpreted [14].

The present study found that T1DM patients had higher levels of anti-CoxV-B IgG than the control group. These findings matched those of Kareem et al. [15], but not those of Bilal et al. [16] who found no differences between anti-CoxV-B IgG in T1DM patients and the control group. A negative anti-CoxV-B IgG test does not rule out a current or recent viral infection. Insufficient IgG antibody levels in the samples may have been acquired too early in the illness development.

Enteroviruses, such as Coxsackievirus, may begin or accelerate the process, leading to clinical T1DM. Viral-induced cytolysis of pancreatic beta-cells may cause direct cell death [16]. Instead, a less aggressive enterovirus infection may cause an inflammatory reaction in the islets, harming beta-cells or triggering an autoimmune response. Enterovirus-induced - cell damage may be produced by molecular mimicry of the islet cell protein due to homologous regions in both enteroviral and islet cell proteins [17].

Positive anti-CoxV-B IgG antibody findings were related to anti-IA2, ICA, and GAD65 in T1DM

patients, compared to negative anti-CoxV-B IgG antibody results. Results of Bilal et al. [16] and Sayah et al. [17], were similar to present the study. T1DM incidence increases following enterovirus outbreaks, suggesting a viral involvement in disease development. Human enteroviruses (HEVs), particularly the Coxsackievirus-B family, have been linked to Beta cell death [18].

Several mechanisms underpinning Coxsackie B4-induced cell dysfunction have been found and explored. Cytosolic infection with Coxsackie B4 may cause cell lysis, revealing self-antigens, and triggering an autoimmune response against cellular antigens. The Coxsackie B4 virus may also activate T lymphocytes, causing direct damage to β-cells. A viral infection may also cause T cells to produce pro-inflammatory cytokines, increasing the body's inflammatory cell activation and infiltration [19].

An extra advantage is an autoimmune response against beta cells caused by structural similarities between viral protein epitopes and cellular antigens. Another theory is that CVB causes alterations in Beta cells, which the immune system mistakenly assaults. An autoimmune response might cause cell death. This might happen if CVB autoantibodies react with a protein present in human islet cells [16].

In conclusions, anti-CoxV-B IgG IgG antibody positivity was associated with autoantibodies related to T1DM. Also, in CVB-T1DM patients, the presence of (anti-IA2, ICA, and GAD65) suggests that CVB viruses may have a role in the development of autoimmune disorders such as T1DM.

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Author contribution

All authors contributed to the study's planning, design, analysis, and interpretation.

Conflict of interest

Authors declare that there is no conflict of interest.

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The Correlation of β/α mRNA Ratio with Clinical and Hematological Parameters in Patients with β-thalassemia Syndrome

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Abstract

Background Thalassemias are a group of genetically transmitted blood diseases characterized by defects in the production of α- or β-chains of hemoglobin called α-thalassemia and β-thalassemia, respectively. One of the common features of β-thalassemia is ineffective erythropoiesis because of the imbalance of globin chain production, which result in increased apoptosis during erythroblast maturation.

Objective To evaluate the ß/α globin mRNA ratio in patients with ß-thalassemia syndrome and to correlate the ß/α ratio with hematological and clinical condition of the patients.

Methods Thirty-five patient samples were collected from Thalassemia Centre of Ibn Al-Balady Hospital; 18 patients with β-thalassemia major and 17 patients with β-thalassemia intermedia. The patients were randomly selected regarding sex, whereas their age ranged from 3 to 17 years. Along with those samples, twenty control leftover samples that were the remaining of samples collected for laboratory investigation, were taken from Al-Kadhimiya Pediatric Hospital, and were age and sex matched with the patients group. The α and β globin chain ration were calculated by the real-time reverse transcription-polymerase chain reaction (qRT-PCR). The β/α -globin mRNA ratio of the samples was measured by the 2−ΔΔCT method.

Results Analysis of the β-globin/α-globin mRNA ratio showed that disease severity increased with a reduction of the ratio. There was a highly significant difference in α level, ß level and ß/α ratio among studied groups with a p value of ($p < 0.001$). The highest level was in control group, followed by that in β-thalassemia intermedia and the lowest was in β-thalassemia major.

Conclusion The severity of ß/α globin chain imbalance showed a significant and negative correlation with the mean corpuscular volume and there was no significant correlation between the ß/α ratio and markers of erythropoiesis in ß-thalassemia major and ß-thalassemia intermedia patients. The ß/α ratio was a good tool for diagnosis in ß-thalassemia major and ß-thalassemia intermedia as compared to control group with high sensitivity and specificity. A ß-chain gene expression was the lowest in ß-thalassemia major followed by ß-thalassemia intermedia as compared to control group.

Keywords Thalassemia, ß/α -globin mRNA ratio

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List of abbreviations: Hb = Hemoglobin, HPFH = Hereditary persistence of fetal hemoglobin, MCH = Mean corpuscular hemoglobin, MCHC = Mean corpuscular hemoglobin concentration, MCV = Mean corpuscular volume, PCV = Packed cell volume, RBC = Red blood cells, WBC = White blood cells,

Introduction

Thalassemias are genetic diseases that are transmitted as autosomal recessive. It is one of the most common single

gene diseases in the world. It results from an imbalance in amount of hemoglobin chains production due to mutations in globin chains [1].

The α- and β-thalassemia syndromes are the most important clinical thalassemia. The α-thalassemia is due to deletion or less commonly to mutation that affect one or more of the duplicated α-globin genes, which is situated on chromosome 16, whereas β-thalassemia is due to point mutations or less commonly to deletion in β-globin gene, which is situated on chromosome 11. These leads to absence or reduction in globin chain synthesis [2].

An imbalanced quantity of β-globin chain production leads to relative increase of α-chain, which is precipitate in erythrocytes, therefore, the clinical phenotype of β-thalassemia related to the excess amount of α-chain, ranging from asymptomatic phenotype the thalassemia minor to severe anemia (thalassemia major). Major thalassemia is transfusion-dependent. It appears in infancy or childhood and it characterized by absence or reduce of normal hemoglobin and severe anemia, enlargement of the heart, liver, spleen, and skeletal deformation [3].

Although the reduction of β-chain production leads to β-thalassemia but the main problem is due to the free α-globin chains, which produce oxidative stress in red blood cells, which leads to hemolysis in red blood cells [4].

The same gene mutation in patients with β-thalassemia have remarkable differences in hematological and clinical symptoms. Several factors like environmental or genetic modifiers are involved in the disease severity of β-thalassemia [5].

Erythropoiesis in β-thalassemia patients is due to proportional increment of free α-globin [6]. The major cause of disease severity is the level of imbalance in the α-globin versus β + γ-globin production ratio rather than the reduction of ß-globin chain production [7].

There is a twofold increment in β-thalassemia trait in the production of α-globin, which have nearly normal hematopoiesis with only mild microcytosis and hypochromia of the red blood cells [8].

The α/β ratio in patients with thalassemia intermedia is typically 3-4/1 because of the presence of reduced amount of β-globin synthesis with γ-globin synthesis to qualify the consequences of excess α-globin production [8].

Patients with β0-thalassemia have marked chain imbalance and it is the underlying basis for their severe phenotype [8].

The unpaired α-globin forms molecular aggregates, will precipitate, and form inclusions, which cause damage to the cell membrane and the membranes of intracellular organelles [7].

One of the most toxic products of unpaired α-chains is hemichromes, which attach to the cell membrane and leads to clustering of band 3, one of the major constituents of cell membrane [7].

The formation of α-chain inclusions occurs early during erythropoiesis and peaks in the polychromatophilic erythroblasts, leading to cellular apoptosis [9].

The severity of β-thalassemia decreased by increased production of fetal hemoglobin (HbF) [10]. The increase in HbF (α and γ chain) is due mainly to selective survival of the cells containing HbF and not to increase synthesis of γ-globin, therefore, there is still excess α chain and ineffective erythropoiesis. Only if there is another mutation such as hereditary persistence of fetal hemoglobin (HPFH) that result in increased synthesis of γ-globin, in this case it will attach to α chain and result in less inclusion and ameliorate the signs and symptoms of thalassemia. The increment in γ-globin synthesis reduces the α/β-chain ratio. As a result, there is improvement in the ineffective erythropoiesis, which cause the disease. That leads to decreased hemolysis, and increased of hemoglobin levels because of improved survival of red cells that contain high levels of HbF [10].

Severe anemia and erythroid hyperplasia, bone marrow expansion and extramedullary hematopoiesis are the causes of ineffective erythropoiesis [11].

β-thalassemia minor causes microcytosis and mild anemia due to decreased HbA synthesis. Patients with minor β-thalassemia have one unaffected β-globin gene and they can produce sufficient amount of hemoglobin without causing significant erythroid hyperplasia. Also, the reduction in hemoglobin level is overcome by an increase in other hemoglobin forms mostly HbA2 [11].

The objectives of this study was to evaluate the ß/α globin mRNA ratio in patients with ß-thalassemia syndrome and to correlate the ß/α ratio with hematological and clinical condition of the patients.

Methods

For all patients and control samples, 0.25 ml of EDTA blood sample was added to 0.75 ml TRIzol for RNA extraction for polymerase chain reaction (PCR).

PCR

Using GoTaq® 1-Step RT-qPCR System, Promega, USA. GoTaq® 1-Step RT-qPCR System used for quantitative analysis of RNA by a one-step reverse transcription-quantitative PCR (RT-qPCR) protocol.

The GoTaq® 1-Step RT-qPCR System act as Reverse Transcriptase and qPCR Master Mix for one-step RT-qPCR quantification.

The GoTaq® 1-Step RT-qPCR System based on the fluorescent DNA-binding dye called BRYT Green® dye, which gives greater fluorescence enhancement when bind to double-stranded DNA (dsDNA) than does SYBR® Green I.

1. RNA was isolated from sample by using TRIzol Reagent.
2. Determine RNA, cDNA yield: Fluorescence Method. To know the quality of samples, a fluorometer was used to know the concentration of extracted RNA or cDNA. For 1 µl of RNA or cDNA, 199 µl of diluted Quantifluor Dye was added after 5 min incubation at room temperature in dark place.
3. Primer preparation: These primers were in a lyophilized form. It then dissolved in a nuclease free water to give a final concentration of 100pmol/µl as a stock solution.

By adding 10 µl of primer stock solution (stored at freezer -20°C) to 90 µl of nuclease free water a working solution of 10 pmol/µl was prepared.

4. Reaction setup and thermal cycling protocol: One Step RT-PCR.
5. Analysis gene expression using pfaffi method.

Relative quantification

Folding =2-ΔΔCT

ΔΔCT =ΔCT Treated - ΔCT Control

ΔCT =CT gene - CT House Keeping gene.

Statistical analysis

Data were analyzed with the statistical package for social sciences software (SPSS); median (range) and frequency and percentages were used to describe continuous and categorical variables, respectively. Independent samples t-test (or Mann–Whitney U-test) and chi-squared test were used to compare continuous and categorical variables, respectively, between two groups. One-way ANOVA was employed for three groups. Correlation analysis was performed with Pearson or Spearman correlation. $P<0.05$ was considered statistically significant.

Results

In this study, the median of α globin gene expression was (0.60) in thalassemia major, (0.87) in thalassemia intermedia and (1.48) in control group. The median of β globin gene expression was (0.12) in thalassemia major, (0.44) in thalassemia intermedia and (1.57) in control group. The median of ß/ α globin genes ratio was (0.24) in thalassemia major, (0.50) in thalassemia intermedia and (1.05) in control group. There was a highly significant difference in α mRNA level, ß mRNA level and ß/α ratio

among studied groups. The p value was (p <0.001); the highest level was in the control group, after that in ß-thalassemia intermedia and then in ß-thalassemia major as shown in table 1.

The ß/α ratio was not significantly correlated to hepatosplenomegaly and frequency of blood transfusion duration in patients with ß-thalassemia major, and patients with ß-thalassemia intermedia (Table 2).

The correlations of ß/α ratio to hematological parameters in patients with ß-thalassemia major revealed a negative correlation with MCV, whereas, in patients with ß thalassemia intermedia, the ß/α ratio significantly correlated to white blood cells (WBC) count, lymphocyte and neutrophil count as shown in table 3.

Table 1. Comparison of alpha gene expression, beta gene expression and ß/α ratio among patients with thalassemia major, thalassemia intermedia and control subjects

Characteristic		Thalassemia major *n* = 18	Thalassemia intermedia *n* = 17	Control *n* = 20	*p*
α gene expression	Median (IQR)	0.60 (0.21) C	0.87 (1.29) B	1.48 (1.04) A	<0.001 K
	Range	0.11-1.20	0.29-3.95	0.68-4.86	HS
ß gene expression	Median (IQR)	0.12 (0.10) C	0.44 (0.67) B	1.57 (0.83) A	<0.001 K
	Range	0.00-0.21	0.13-3.02	0.74-6.11	HS
ß/α ratio	Median (IQR)	0.24 (0.18) C	0.50 (0.20) B	1.05 (0.27) A	<0.001 K
	Range	0.00-0.54	0.32-0.80	0.90-1.20	HS

n: number of cases, IQR: inter-quartile range, K: Kruskal Wallis test, HS: highly significant at p ≤0.01, Capital letters (A, B and C) were used to indicate the level of significance following post hoc Dunn's test so that similar letters indicate no significant difference whereas, different letters indicate significant difference

Table 2. Correlations of ß/α ratio to splenomegaly, hepatosplenomegaly and frequency of blood transfusion duration in patients with ß-thalassemia

Characteristic	Thalassemia major		Thalassemia intermedia	
	r	*p*	*r*	*p*
Splenomegaly	-0.055	0.828 NS	-0.241	0.351 NS
Hepatosplenomegaly	0.055	0.828 NS	0.057	0.829 NS
Frequency of blood transfusion	-0.242	0.333 NS	0.329	0.198 NS

r: correlation coefficient, NS: not significant at p >0.05

Table 3. Correlations of ß/α ratio to hematological parameters in patients with ß-thalassemia

Characteristic	Thalassemia major		Thalassemia intermedia	
	r	*p*	*r*	*p*
Hb	-0.460	0.055 NS	-0.135	0.605 NS
PCV	-0.388	0.111 NS	-0.021	0.936 NS
MCV	-0.478	0.045 S	0.091	0.729 NS
MCH	-0.170	0.499 NS	-0.024	0.927 NS
MCHC	-0.201	0.424 NS	-0.297	0.246 NS
RBC	-0.318	0.199 NS	-0.059	0.823 NS
Ferritin	0.238	0.342 NS	-0.042	0.873 NS
Platelet	-0.058	0.820 NS	-0.088	0.737 NS
WBC	-0.363	0.139 NS	0.486	0.048 S
Lymphocyte	-0.248	0.321 NS	-0.494	0.044 S
Neutrophil	0.291	0.241 NS	0.570	0.017 S
Monocytes	-0.243	0.331 NS	-0.329	0.197 NS
Eosinophils	0.048	0.849 NS	0.229	0.378 NS
Basophils	*	*	0.358	0.158 NS

r: correlation coefficient; NS: not significant at $p > 0.05$; S: significant at $p \leq 0.05$; *: basophil is constant in patients with beta thalassemia major, Hb: hemoglobin, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RBC: Red blood cells, WBC: White blood cells.

Discussion

The ß/α globin chain imbalance is central to the ineffective erythropoiesis in β-thalassemia, as it triggers a sequence of events that lead to premature cell death [12].

There was a highly significant difference in α gene expression, ß gene expression difference and ß/α ratio level among studied groups, with p value of ($p<0.001$); the highest level was in control group, followed by β-thalassemia intermedia and then by major β-thalassemia.

This result was comparable with Ranjbaran et al. [3], Watanapokasin et al. [13] and Ahmedy et al. [14] studies, which found that α-globin gene expression was higher in thalassemia intermedia group versus thalassemia major, while β-globin gene expression was lower in thalassemia major group compared with thalassemia intermedia ($p<0.001$) and β/α-globin genes ratio was higher in control group compared with both thalassemia groups ($p<0.001$).

The severity of β-thalassemia depends on the degree of imbalance between ß and α-chains and the amount of the unpaired α-chain. So, the factors that reduce the extent of chain imbalance and the extent of α-chain excess in the red cell precursors will affect the phenotype [15]. The severity of β-thalassemia increased when the ß/α ratio decreased. As this result in precipitation of extra α-chain in erythrocyte membrane, which eventually result in ineffective erythropoiesis and hemolysis. Moreover β/α ratio was significantly higher in control group compared to both thalassemia groups, the decline in this ratio is mainly because of α-chains rather than decrease β-chains formation and the major pathophysiological basis of β-thalassemia is a free α-globin chains which produce oxidative stress in red blood cells, which leads to ineffective erythropoiesis and erythrocytes hemolysis [16].

In this study, the median of α, ß-globin gene expression and the median of ß/α globin genes ratio in thalassemia major, thalassemia intermedia and control group were comparable to other studies [3,14,16].

The ß/α ratio was not significantly correlated to splenomegaly, hepatosplenomegaly and

frequency of blood transfusion duration in patients have β-thalassemia major and β-thalassemia intermedia. No similar studies were found. This may be due to small size sample.

The ß/α ratio was negatively correlated to MCV in patients have β-thalassemia major. This was comparable to other study [2], the ß/α ratio correlates with the severity of anemia. whereas, in patients with β-thalassemia intermedia, the ß/α ratio were significantly correlated to WBC count, lymphocyte count and neutrophil count. No similar result was found. This may be due to infection because thalassemia patients more susceptible to infection

In conclusions, the severity of ß/α globin chain imbalance showed a significant and negative correlation with MCV and there was no significant correlation between the ß/α ratio and markers of erythropoiesis in ß-thalassemia major and ß-thalassemia intermedia patients; including clinical findings, frequency of blood transfusion and most of the hematological parameters in the studied samples. The ß/α ratio was a good tool for diagnosis in ß-thalassemia major and ß-thalassemia intermedia as compared to control group with high sensitivity and specificity. A ß-chain gene expression was the lowest in ß-thalassemia major followed by ß-thalassemia intermedia as compared to control group.

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Author contribution

Dr. Yousif: Samples collection, laboratory work and writing the draft of the article. Dr. Al-Mamoori: helped in data analysis and revising the article manuscript.

Conflict of interest

Authors declare that there is no conflict of interest.

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Is Locked Compression Plate Better than Limited Contact Dynamic Compression Plate in Treatment of Closed Middle Third Radius and Ulnar Fractures in Adults: A Short-Term Comparative Study

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Abstract

Background Forearm bone fracture is a commonly encountered fracture. The inception of locking compression plate (LCP) has revolutionized fracture management. With their dramatic success for articular fractures, there is a speculation that they might be more appropriate for diaphyseal fractures as well.

Objective To compare internal fixation of closed, middle third forearm fractures with LCP and limited contact dynamic compression plate (LC-DCP) in adults with respect to union rate, implant failure, functional outcome, and infection rate.

Methods Twenty-two patients with closed, middle third fractures of both the forearm bones were involved in this prospective, randomized, controlled study, which took place between February 2019 to January 2021. They were segregated into two groups based on open reduction and internal fixation with LCP (n=11) and with LC-DCP (n=11). Postoperative follow-up intervals of 1, 2, 6 weeks and 3, 6 months. The patients were assessed for implant failure, fracture union and function outcome of Andersons' criteria to assess union, forearm rotation, and wrist flexion-extension, and disabilities of the arm, shoulder and hand (DASH) score for patient related outcome at the latest follow up.

Results The mean age of the patients was 30.9 years (range 19-47 years) with mean follow up about of 2 years. The union rate in LCP group was (100%) whereas in LC-DCP was (81.8%), the p value was (0.4), which is not statistically significant. The p value for Quick DASH score and Anderson' criteria were (0.8 and 0.43), respectively which is also not statistically significant. No incidence of implant failure in both groups.

Conclusion Although LCP is an effective treatment alternative and may have a subtle edge over LC-DCP in the management of these fractures, their supremacy could not be certified. We deduce that surgical planning and expertise rather than the choice of implant are more pivotal for outstanding results.

Keywords Limited contact dynamic compression plate, locking compression plate, closed, middle third fractures, both bones of forearm

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List of abbreviations: AP = Antero-posterior, DASH = Disabilities of arm, shoulder, and hand, LC-DCP = Limited contact dynamic compression plate, LCP = Locked compression plate, ORIF = Open reduction and internal fixation

Introduction

Diaphyseal fractures involving the radius and ulna, so called ''both bone'' or ''double-bone'', forearm fractures are

common orthopedic injuries. These injuries can cause significant loss of function if inadequately treated. As the upper extremity serves to position the hand in space, loss of forearm motion and/or muscle imbalance resulting from a poorly treated fracture can be particularly debilitating. Preservation of the anatomic relationships of the proximal and distal radioulnar joints as well as the interosseous space is critical to preserving function [(1).]

It is essential to regain length, apposition, axial alignment, and normal rotational alignment while treating diaphyseal fractures of the radius and the ulna to gain good range of pronation and supination. The chances for the occurrence of malunion and non-union are greater because of the difficulties in reducing and maintaining the reduction of two parallel bones in the presence of the pronating and supinating muscles, which have angulatory as well as rotatory influences [(2)].

The objective of this study was to compare internal fixation of closed, middle third forearm fractures with locking compression plate (LCP) and limited contact dynamic compression plate (LC-DCP) in adults with respect to union rate, implant failure, functional outcome, and infection rate.

Methods

A prospective comparative randomized study was conducted from February 2019 to January 2021 including the follow up, at the Department of Orthopedic Surgery at Al-Imamein Al-Kadhimein Medical City. Twenty-two patients had been evaluated, five patients were female and seventeen were male, who had closed radius and ulna middle third fractures. The patient sample divided randomly by choosing every other patient into two groups: group 1 (11 patients) fixed by LCP, and group 2 (11 patients) fixed by LC-DCP.

In both groups, the radius fixed by volar (Henry) approach, which is offers good exposure of the whole length of the radius, and ulna fixed by direct subcutaneous approach.

The patients were collected and evaluated in Outpatient and the Emergency Department in our hospital and patients referred from other hospitals. All patients approached by the same surgeon team, and followed postoperatively at 1-week, 2-week, 6-week, 3-month, and 6-month. The current assessment was done based on history of the patients, clinical examination, and radiography.

A brief information about the surgery, implant, and the enrollment in the study was discussed with each patient and verbal agreement was taken.

Inclusion criteria

- Skeletally mature patients (closed physis).
- Both forearm bones, closed, middle third fractures.
- Acute presentation within 14 days of injury.
- (Transverse, short oblique) radius and ulna fractures.
- Low energy trauma.

Exclusion criteria

- Open fracture.
- Previous fracture in same limb.
- Pathological fracture.
- Associated distal radioulnar joint dissociation, elbow dislocation.
- Osteoporotic bone.
- Patients not fit for surgery.
- Neuropathic patient.
- Multiple traumas.
- Deformed radius and ulna.
- Vascular or neurological injury.
- Associated comorbid diseases (renal failure, uncontrol diabetes HbA1c more than 7.5%, heart failure).

The patients were prepared for the nearest elective surgery list (all were operated within the first 14 days of injury) after optimization of all the facilities and the patient`s general condition and performing all the laboratory investigations. At the day of surgery patient was admitted to surgical unit of orthopedic and ceftriaxone 1 g vial given intravenously within 1 hour before skin incision, after checking the

allergy condition to the drug. A volar Henry approach was utilized to fix the fractures of radius. Ulna was exposed through an incision over its subcutaneous border and its dorsal surface was plated. In both groups we started with fixation of the radius then the ulna. And each bone fixed by six cortices in each fragment. The patients in both groups were kept in the Hospital Orthopedic Ward under observation, active finger exercise encouraged immediately after surgery, paracetamol 500 mg vial (three times per day), ceftriaxone vial 1gm intravenously at 8 and 16 hours postoperatively, initial plain radiograph was taken before discharge to assess reduction, and the limb kept in elevation by arm sling for 14 days.

Simple oral analgesia (paracetamol) on need was prescribed for all patients. During this period, elevation, gentle finger motion, active and passive, together with shoulder motion can be started.

Follow up criteria for both groups

All patients in this study were followed-up in outpatient clinic after 1 week to change dressing and the wound inspected for signs of infection.

Then followed at 2nd week to inspect and assess the wound healing and stiches removed. Active assisted range of motion exercises, including gentle forearm rotation, elbow flexion and extension begin. Lifting and resisted exercises are restricted until radiographic signs of healing appear.

Then followed in 6th week and 3rd month for radiological assessment (AP and lateral plain radiograph was taken), for union and implant failure, and for clinical assessment of forearm rotation movements. Further followed up in the 6th month postoperatively for clinical and radiological union assessment, implant failure and for functional outcome assessment using Quick Disabilities of the Arm, Shoulder and Hand (DASH) scoring system [3] and Anderson et al. criteria [4]. All patients in both groups were followed up for the following parameters:

- ***Union***: assessed according to Anderson et al. Criteria [4]. Osseous healing was designated radiologically in AP and lateral radiographs. And absence of pain and tenderness at fracture site dictated the achievement of clinical healing.
- ***Functional outcome of forearm rotation and wrist flexion-extension***: assessed by Anderson et al. criteria [4]. Forearm Rotation and wrist flexion-extension measured using goniometer.
- ***Implant failure***: (plate breakage) or screws (pullout or breakage).
- ***Physical function and functional outcome***: assessed by Quick DASH score.
- ***Infection***: whether superficial infection (not reaching bone and joint and could be treated as outpatient with oral or intravenous antibiotics) or deep infection.

Results

All patients achieved union by 6th month interview (2 patients in group 2 developed delayed union), the same 2 patients have had superficial infection treated by oral antibiotics and changing dressing (Figures 1 and 2).

No patient in both groups had implant failure or loss of fixation. No patient in both groups had poor results with Anderson functional criteria or DASH score (Table 1).

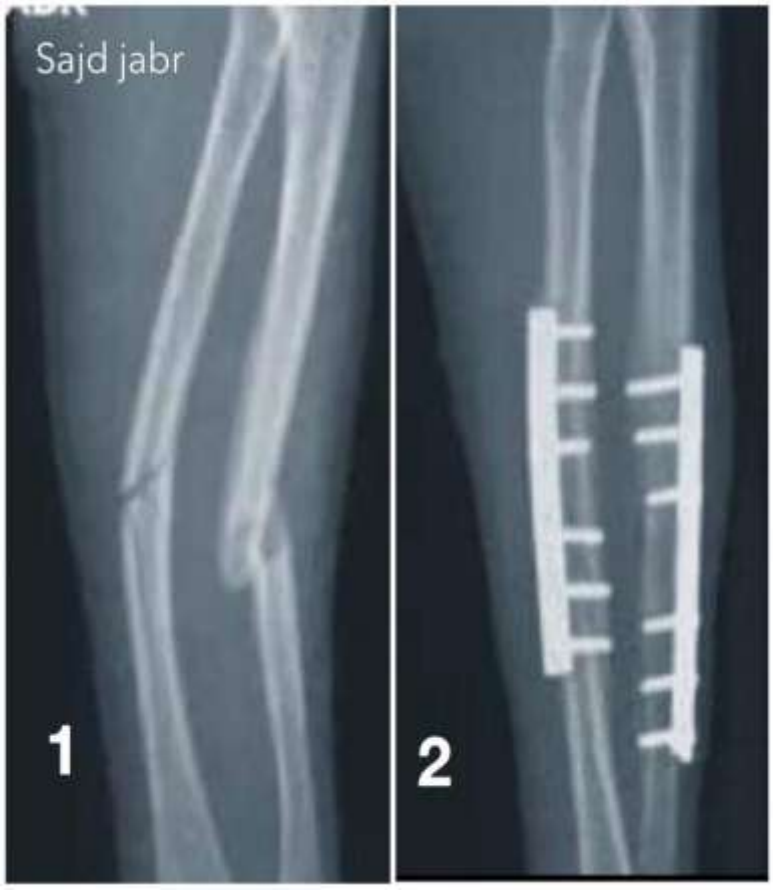

Figure 1. Pre - and post-operative plain radiograph of a patient in group 2 where (1) is a plain radiograph of forearm showing both bone forearm fracture and (2) is 6 months after fixation with LCP with complete obliteration of the fracture line

Table 1. Number and percentage of follow up parameters

Parameter	Subdivision	LCP N (%)	LC- DCP N (%)	P value
Union	Perfect	11 (100%)	9 (81.8%)	0.4
	Delayed	0 (0.0%)	2 (18.2%)	
Functional outcome	Excellent	7 (63.6%)	4 (36.4%)	0.43
	Satisfactory	3 (27.3%)	5 (45.5%)	
	Unsatisfactory	1 (9.1%)	2 (18%)	
DASH score	Excellent	1 (9.1%)	1 (9.1%)	0.8
	Good	9 (81.8%)	8 (72.7%)	
	satisfactory	1 (9.1%)	2 (18.2%)	
Superficial infection	Yes	0 (0%)	2 (18.2%)	0.4
	No	11 (100%)	9 (90.9%)	
Failure of fixation or implant	Yes	0 (0%)	0 (0%)	1.00
	No	11 (100%)	11 (100%)	

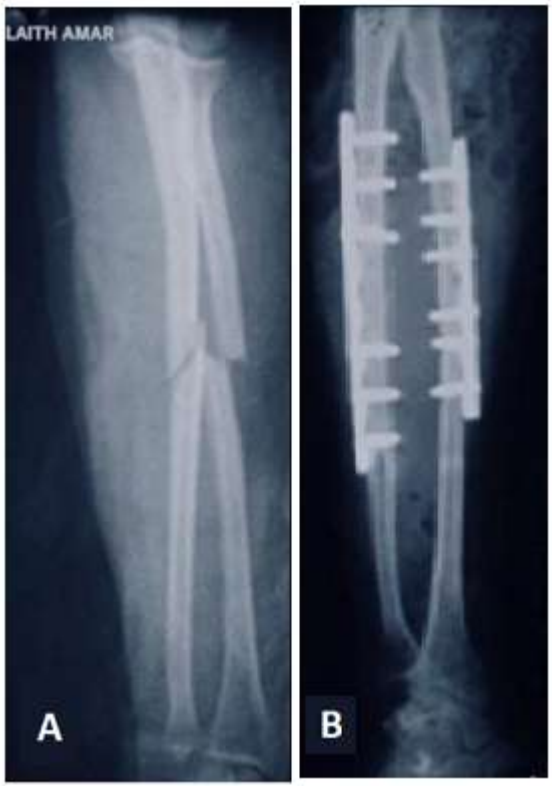

Figure 2. A radiograph of patient from group 2 who treated by open reduction and internal fixation (ORIF) with LC-DCP. With (A) represents mid shaft fracture of both radius and ulna preoperatively and (B) represents postoperative fixation of both bones by LC-DCP after 6 months showing complete union

Discussion

Fracture of both bones of the forearm are relatively common injuries, which can challenge the treating physician. Healing occurs after closed treatment but malunion with resultant decreased rotation of the forearm, is common and has been associated with poor outcomes. Rotation of the forearm is a complex interaction between the radius and the ulna and restoration of movements depend upon both an accurate reduction of fractures and early initiation of postoperative movements. Loss of rotation impedes function of the upper limb and activities of daily living [5].

Open reduction and plate fixation has been the standard treatment of adult diaphyseal forearm fractures [6], but the most effective type of plate fixation for diaphyseal fractures of forearm bones has not been well defined [7].

Locked plates do not rely on frictional force between the plate and the bone to achieve compression and provide absolute stability. Thus, the local blood supply under the plate to be preserved [8], thereby leading to superior bone healing and minimal complications. It has been proved to be valuable in situations like osteoporosis, comminuted fractures, osteotomy, complex intraarticular fractures or fractures in close proximity to the joints [9].

The sample size in this study was (22) patients. The mean age included in this study was about 30.9 years, (77.3%) males and (22.7%) females, of the total patients, right hand affected in about (73%) while (23%) got left side fracture, these variables (age, gender, hand dominancy) are normally distributed in our community because of most workers are active males from middle age group with right hand dominancy, which is comparable to Gill et al. study [10] for

comparison between LCP and LC-DCP in diaphyseal fracture of radius and ulna in adults. The mean age was 32 years, male (74%), female (26 %), right hand (57%) and left hand (43%), which is also comparable with Saikia et al. [11] study. They were (25) males (70%) and (11) females (30%), with an average age of (30.5) years.

These two studies states that females tend not to have as many radius and ulna fractures as the male counterparts because they tend not to partake in the same level of high velocity and sport activity.

Regarding to union rate, this study had 2 patients with delay union (2 of 11) in group 2 (18%) and union occurred without resorting to any secondary procedure. While in group 1 we did have (100%) union, (11 of 11) patients, and there was no significant statistical difference between LCP group and LC-DCP group.

These results were comparable to Gill et al. study [10], which had (88%) union, (8%) delay union and (4%) nonunion in 26 patients with diaphyseal radius and ulna fracture fixed by LC-DCP, and (96%) union and (4%) delay union in 26 patients with closed diaphyseal radius and ulna fracture fixed by ORIF with LCP. The difference between two groups was not significant.

In Saikia et al. study [11], the sample size was (36) adult patients with closed diaphyseal radius and ulna fracture, had (100%) union with (18) patients fixed with LCP, and (94%) had union, and (6%) had delay union with (18) patients fixed with LC-DCP. The difference was not significant and could not prove the superiority of LCP because He suppose the quality of reduction and stability of fracture which determine the union rate. Leung and SP Chow prospective study, locking compression plate in the treatment of forearm fractures: reported that the LCP is an effective bridging device used for treating comminuted fractures, but for treating simple fractures its superiority over conventional plating is yet to be proven. Reddy et al. [12] reported the mean time of union for the forearm fixed with LCP was found to be (18 weeks) in comparison to (16 weeks) for the LC-DCP group and this result is not significant. Vishwanath et al. [13] study consists of 50 cases of fracture both bone forearm fractures. All cases were treated operatively with 3.5 mm LC-DCP and reported (98%) union rate and concluded that LC-DCP can be considered the best mode of treatment for closed diaphyseal fractures of both forearm bones because it minimizes vascular damage to the plated bone segment.

More than three studies as shown above agreed with the result in this study as no significant difference between LCP and LC-DCP for closed diaphyseal fracture of the radius and ulna. This may explain as the quality of reduction, stability of fracture, proper application of the biomechanical principles of plating and not the type of plate, which determine the union outcome.

Regarding infection, in this study, there was no infection in LCP group, whereas two patients in group (2) had superficial infection within the first week which subsided with antibiotic and dressing. Both patients ended up with delayed union (>6 months), both of them were smokers and one was diabetic. There is no significant statistical difference between LCP group and LC-DCP group. This result is comparable with Gill et al. [10] reported (88%) no infection, (8%) superficial infection and (4%) deep infection in LCP group, and (3%) superficial infection and (3%) deep infection in LC-DCP group. Saikia et al. [11] had (6%) deep infection in LCP group and (11%) superficial infection in LC-DCP group. And these results are not statistically significant. Also, in Leung and Chow [6] and Mohamed Shakeeb et al [14] reported that no significant difference. This could be explained upon the fact that the infection caused by patient factors like smoking and comorbidities and not related to implant.

Regarding functional outcome through 6th month follow up interval using Anderson et al. criteria [4], the results of this study showed no significant difference between two groups, this thesis reported (63.6%) excellent outcome, (37.5%) satisfactory, and (33%) unsatisfactory in (group 1), and (36.4%) excellent outcome, (62.5%) satisfactory, and (66.7%) Unsatisfactory outcome in (group 2).

And this result was comparable to Saikia et al. [11] who reported (88%) excellent outcome, and (12%) satisfactory outcome in LCP group while reported (88%, 6%, 6%,) excellent, satisfactory, and unsatisfactory, in LC-DCP group respectively. Gill et al. [10] in the LC-DCP group, (57%) excellent results, (32%) satisfactory, (7%) unsatisfactory and (4%) was recognized as failure. In the LCP group, (77%) excellent, (19) satisfactory and (4) unsatisfactory. These results are better for LCP but not able to statistically prove better results of LCP. Vishwanath et al. [13] reported LC-DCP gives excellent functional results in most of the patients. Leung and Chow [6] reported the functional outcome of LCP gives excellent results.

Reddy and Reddy [15] reported the functional results were almost same in both groups, in spite of different rates of radiological union.

Regarding functional outcome through 6th month follow up interval using DASH score, the results of this study showed (50%) excellent outcome, (52.9%) good outcome, and (33.3%) satisfactory outcome in (group 1). While in (group 2) the results showed (50%) excellent outcome, (47.1%) good outcome, and (66.7%) satisfactory outcome. The result of this study showed no significant difference between two groups. These results were comparable with Gill et al. [10] and Saikia et al. [11], these studies showed no significant difference between LCP and LC-DCP groups. Henle et al. [16] compared LCP with the LC-DCP when used for "bridging technique" and "axial compression." fixation and concluded that the LCP did not demonstrate any superiority over LC-DCP in terms of functional or clinical outcomes. Mohamed Shakeeb et al. [14] reported that the outcome is determined by using the proper principles of plating, and the LCP gives better results in comminuted both bones forearm diaphyseal fractures in comparison to dynamic compression plate even though cannot prove better results overall forearm fractures. This could explain that early mobilization prevents soft tissue contracture, muscular tethering and improves the vascularity.

Regarding the implant failure (plate breakage) or screws (excursion or breakage). This study showed no implant failure in both LCP and LC-DCP groups. Saikia et al. [11] reported no implant failure, but Gill et al. [10] had (1 of 28) case with loosening of LC-DCP. Vishwanath et al. [13] reported (2%) implant failure in LC-DCP.

In conclusion, although LCP is an effective treatment alternative and may have a subtle edge over LC-DCP in the management of these fractures, their supremacy could not be certified. We deduce that surgical planning and expertise rather than the choice of implant are more pivotal for outstanding results.

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Author contribution

All the authors contribute to this article by their patients and participate to the data collection and statistical analysis.

Conflict of interest

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