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A Review Analyzing In Vivo and In Vitro Testing Models on Nerve Conduits of the Peripheral Nervous System

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Abstract

The gold standard method for nerve reconstruction involve the use of autologous graft, however, major drawbacks included limited availability, donor-site morbidities and requirement of multiple surgeries. Researchers worldwide had aimed to produce alternative tissue-engineered synthetic nerve conduits, but development had been slow, with only four FDA (US Food and Drug Administration) approved conduits for human subjects in the past 50 years of research. This slow progress may potentially be related to the lack of standardized guideline for nerve conduit testing. This review aims to summarize the methodologies used in the testing of nerve conduits in vivo and in vitro. The review demonstrated a lack of consensus and consistency in the study methodologies, including various measures of functional assessment, over 8 different types of animal species, 17 peripheral nerves and varied gap lengths ranging between 1 mm and 90 mm. In vitro models demonstrate more consistencies in testing models, but have been discarded in recent years for functional nerve testing, and had been employed for preliminary testing in nerve toxicity and compatibility instead. This study emphasizes the urgent need for a more standardized approach for in vivo testing, and the need to re-utilize in vitro studies for functional testing purposes.

Key words: nerve conduits; tissue engineering; peripheral nerves; testing methodology

Introduction

Tissue engineering has advanced as an integrative field, which incorporates cells, growth factors, biomaterials and engineering to produce an artificial section or system capable of replacing damaged human tissue or to improve its functional effectiveness [(1)]. The nervous system is one of the numerous areas in which tissue engineering is focusing on; nerve conduits being a crucial element of that advancement [(2,3)].

A nerve conduit is a guide tube manufactured from either natural or synthetic materials. It aims to restore sensitivity to nerve gaps caused by trauma, degenerative disease or tissue loss due to tumor resection [(4)]. Autologous nerve conduits are the current gold-standard tool [(5)] for repair of injured or diseased nerves, the sural nerve being the most commonly used for nerve grafting in humans [(6)]. However, complications such as sensory loss, neuroma and scar formation [(4)] may arise following peripheral nerve harvesting. Due to the resulting donor site morbidity and graft mismatch, an alternative is currently needed. Thus, the development of artificial nerve conduits began to progress toward replicating a nerve that may match the former's functional capabilities. In that context, nerve conduits' function is being tested throughout research for the last 50 years by varying techniques and methods, yet, a standard testing method does not exist [(7,8)].

Nerve tubulation (conduits) was first introduced in the 19th century by Gluck; he has proposed the use of nerve conduits in 1880 whereby he employed the use of a bone as a tube for nerve repair [(9)]. Gluck has adapted his idea from

Neuber who had used a bone tube in 1879 to serve as a resorbable wound drain [9]. In current practice, the US Food and Drug Administration (FDA) and the conformity European (CE) approved the clinical use of four artificial nerve conduits; two are type 1 collagen nerve conduits and the other two conduits are synthetic polyester-based [10,11].

This review aims to summarize the existing testing methodologies of artificial nerve conduits in the setting of both in-vitro and in-vivo models and to analyze the outcome of these methods in order to attain a standardized method of research for future nerve conduit studies in the peripheral nervous system.

Review

In-Vivo models

The use of animal models for nerve conduit testing flourished in the past decade producing abundant volumes of published studies. A systematic review conducted by Angius *et al* in 2012 analyzed the methodologies of more than 416 published in-vivo nerve conduit studies and concluded there was genuine lack of consensus and consistency in researchers' choice of methods [12]. The variability of methods included the choice of animal model, tested nerve, gap length, and assessment tool.

Choice of animal

The most popular choice of animal was rats, which accounted for up to 70% of all in-vivo studies [12]. The advantages of using rats included low maintenance cost, resilience to surgical intervention and infections, availability, and production of consistent assessment outcomes [13-15]. However, the drawbacks included the relatively small gap length compared to common human nerve lesions, the difference in neurophysiology to humans i.e., nerve axotomy produces full recovery in rats but not in human nerves and nerve regeneration is slower in humans [12,16,17]. Furthermore, there are different species of rats which have unknown variations in their physiological response to foreign materials for nerve regeneration [12].

The remaining 30% of animal models were accounted for by mouse, rabbits, dogs, cats and monkeys, with a few scattered studies on sheep and guinea pigs [12] (Fig. 1).

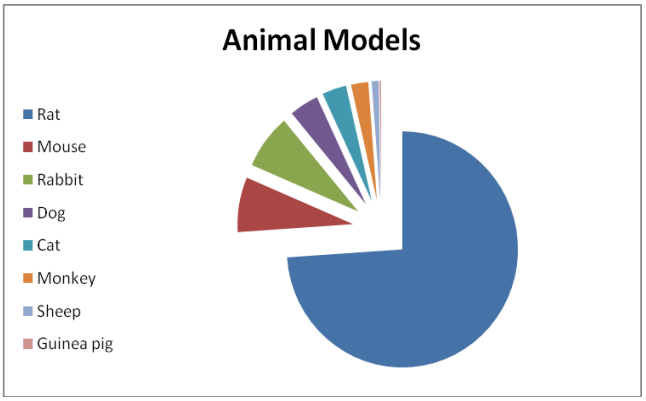

Fig. 1. Pie chart illustrating the types of animal model used in 'in vivo' studies

The mouse model was used in 7.5% of all studies and shared similar advantages and disadvantages to the rat model. One unique advantage in the mouse model was the ability to genetically modify mouse to allow imaging of nerve regeneration of fluorescent-induced axons [18,19]. The major disadvantage of mouse model was its limited gap length of less than 13mm [12].

The rabbit model had been one of the more frequently used models amongst the larger animals (up to 7.5% of studies). The rabbit model facilitated testing of larger nerve gap lengths and produced reliable results from neuromorphometric and electrophysiological testing methods [12]. However, its disadvantages included cost, difficulty of care, limited molecular probes for mechanistic analysis and most importantly, the difference in anatomy e.g., hind limb muscle in rabbits functions to hop, this may reduce its strength for human clinical trials [12]. Nerve studies on dogs and cats also allowed large testing nerve gap, and commonly produced reliable neuromorpho-metric analysis [12]. One major advantage in the use of dogs was the ability to train the animal for functional motor and sensory analysis, however, major drawbacks, together with cats,

included maintenance cost, ethical concerns in their role as domestic animals, and the lack of molecular probes present for mechanistic analysis [12].

There use of larger animals such as monkeys, sheep and guinea pigs in nerve conduit testing were less common (approximately 10, 4 and 1 reported cases, respectively). These animals allowed larger nerve gap length up to 60mm to be tested. However, these studies were restricted due to high cost and limited range of assessment tools available, including difficulties in training these species for functional testing compared to dogs [12]. Although the study of nonhuman primates i.e. monkey, would provide presumably the most reliable outcomes for a step toward human trials, recent reports from the Institute of Medicine had pledged their disagreement to nonhuman primates testing [20,21].

Overall, the selection of the animal type for clinical trials was essential, and researchers must consider the cost, availability, ethical issues and importantly, the physiology of the species e.g. lifespan, inter-variation of the species, susceptibly of infection and ability to withstand surgical interventions [22,23].

Furthermore, compatibility of the neuro-physiology of the animal species to the human being must also be considered i.e., neuromicrostructure, inflammatory response, degeneration process (Wallerian), and regeneration capacity [24,25]. It is important to make aware that the testing model used will depend on the experimental question, thus most authors would agree that no single testing model will fit all, nevertheless, the call for a more standardized methodology and guidelines will aid research forward [13,23,26]. In addition, strict adherence to national regulation in animal-testing policies is vital [27].

Type of peripheral nerve and length of nerve gap tested

The most commonly tested nerve was the sciatic nerve, accounted for over 70% of all studies [12]. The popular use of the sciatic nerve was likely to be due to its relatively anatomical accessibility and size compared to other peripheral nerves. The peroneal, tibial and facial nerve accounted for approximately 5-7% of the studies. A total of 17 different types of peripheral nerves that had been used for nerve conduits studies [12] (Fig. 2).

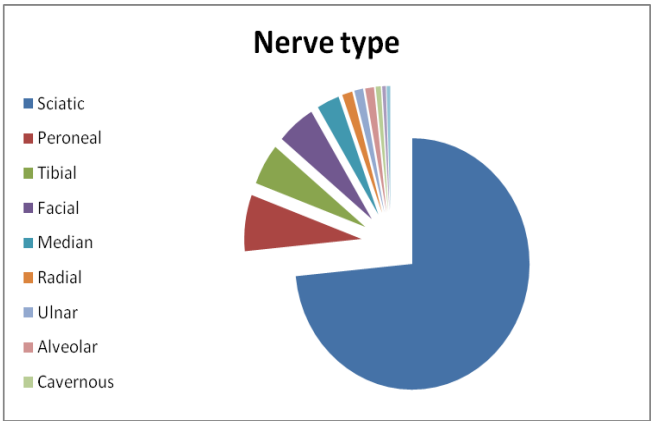

Fig. 2. An illustration showing the types of peripheral nerves used in 'in vivo' studies

Small volumes of individual studies used the median, radial, ulnar, alveolar, cavernous, saphenous, hypogastric, sural, optic, phrenic, recurrent laryngeal lingual and femoral nerves [12]. Overall, the selection of nerves was likely to be governed by resources, animal variability, and most importantly, intended purpose of the clinical trial.

As previously described above, the length of nerve gap examined were influenced greatly by the selection of the tested animal: rats 1-50 mm, mouse 2-13 mm, rabbits 2-50 mm, dogs 10-90 mm, cats 1-50 mm, monkeys 1-50 mm, pigs 8mm and no nerve gap was examined in the sheep study (Table 1).

Table 1: Table illustrating gap lengths (mm) used in 'in vivo' studies

Nerve gap length	
1-5 mm	29.5%
6-10mm	54%
11-15mm	14%
16-20mm	7%
24-30mm	3.4%
40-90mm	3.6%

The ideal nerve gap length studied would mimic distances commonly encountered in human nerve injuries, which vary tremendously. In most studies, the selection for gap lengths were >2 mm, which were decided upon the concept of critical length i.e., gap distance which regeneration would not occur unless nerve grafting or bridging occurs. Studies that conducted testing gap lengths < 2mm were not clear in the reason behind their selection. The range of gap lengths tested was from 1mm to 90mm. The most frequently used gap lengths were small distances of 1-5 mm and 6-10 mm, which accounted for 80% of all studies, followed by intermediate lengths of 11-15 mm, 16-20 mm and 24-30mm (25% of cases). Larger gap lengths of 40-90mm were less commonly tested.

Assessment tool for testing nerve conduit

There were a vast number of available testing tools used to assess nerve recovery and function (Table 2).

Table 2. Illustrates the common types of assessment methodologies in nerve conduit studies (in vivo)

Testing Methodologies	
Histological Analysis	80%
Neuromorphometric Analysis	50%
Electrophysiological	40%
Functional Analysis	27%
Immunohistological Analysis	25%

Most studies used more than one testing method [(28)], but there were great inconsistencies in their selection, with little consensus on the definitive testing tool. Furthermore, studies rarely explained or rationalized their choice of testing tool. This inconsistency could not simply be explained by the differences in experimental outcome or the influence of the type of animal used i.e., dog models were ideal for observational functional outcomes [(29-31)].

The most common assessment tool was qualitative histological analysis, which was present in around 80% of studies, followed by neuromorphometric analysis and electro-physiological analysis, which was present in 40-50%. A quarter of the studies utilized functional analysis and immunohistological analysis as their assessment tool. Other less common methods included gene analysis (RNA, DNA expression), stain analysis (retrograde labeling, BrdU staining), observational analysis (fast axonal transport assay, fiberscopic), muscle analysis (weight, contraction test, morpho-metric analysis) and imaging analysis (radiological, ultrasound) [(32-34)].

Histological and neuromorphometric analysis consistently reported myelinated-fiber count, nerve-fiber count, axon diameter, myelin thickness and g-ratio as endpoints, but failed to comment on which portion of the nerve was examined i.e. distal, central or proximal part. Furthermore, method of tissue processing were not often discussed i.e. tissue collection and sampling procedures. Electrophysiological analysis commonly measured the amplitude and latency of compound muscle action potentials (CMAP) or sensory nerve action potentials (SNAP), and occasionally, centrally recorded somatosensory evoked potentials. However, there were no consistencies amongst studies in their stimulation and recording parameters, as well as the location of nerve stimulated.

The majority of studies that tested functional analysis measured motor function, which included gait studies (static or dynamic), strength measurement (grip strength), and task assessments (object transfer). A common standardized motor test was the 'sciatic function index' that measured functional gait of rat (sciatic nerve) by standardized walking tracks [(35)]. There are over 20 different types of cellular markers for immunohistological analysis. Other less common used assessment tools included gene expression, muscle integrity, and imaging i.e., one study used ultrasound imaging.

This variability in testing methods potentially

highlights the poor communication amongst researchers, suboptimal available testing methods, and complexity and heterogeneity of nerve testing. However, we believe the utilization of a combination of testing methods within studies appeared constructive and logical, as it provided broaden ranges of analytical data. The use of the combination of histological, neuromorphometric or electro-physiological analysis provided valuable information in the different aspects of neurophysiology in nerve regeneration [33].

Functional analysis provided gross scale nerve recovery and was particularly testing models using dogs. Advancing techniques with cellular markers in immunohistological analysis offered targeted analysis of nerve regeneration. Therefore, the ideal testing methodology should in theory target a range of parameters; nevertheless, a standardized guideline, potentially containing various ranges of assessment tool, is essential to formulate a more structured approach to in vivo nerve conduit testing [36,37].

Nerve conduit composition and reconstruction

The type of nerve conduits used would be the tested variable in studies and would influence the assessment tool used, and to a degree influence the selection of animal model, nerve type or gap length. This further highlighted the complexity of selection in nerve conduit testing. There were more than 70 different synthetic nerve conduit materials being tested, broadly categorized into synthetic biodegradable, synthetic non-biodegradable and semi-synthetic materials derived from biological source, e.g., collagen, chitosan and silk.

Furthermore, methods of material extraction, processing, and scaffold integration for biological materials e.g., collagen, in the construction of semi-synthetic conduits, greatly varied between studies [12].

This demonstrated another inconsistency in testing methodologies. It was important to note that the differences in nerve conduit composition and reconstruction techniques used would be variable-tested, therefore would be independent from the selected testing method [38].

In-Vitro model

The testing of nerve conduit in recent years had favored in vivo animal studies to in vitro models, with the majority of in vivo studies functioning to assess biological safety and biocompatibility rather than functional outcome. At present, in vitro studies fail to mimic in vitro nerve environment, thus fail to assess immune response or tissue reaction secondary to vascularisation, oxygen supply and waste elimination present in in-vitro studies ([12]. Nevertheless, in vivo nerve conduit testing plays an essential part in clinical approval.

All biomaterial have to pass in vitro and in vivo tests to get FDA and CE approval, there are only four type of nerve conduits were approved (2 collagen- and 2 synthetic- polyester based conduits). The International Standard Organization in ISO 10993-11 put the criteria for biological safety but not for the functional outcome.

In our literature search, there were relatively less published studies on in vitro models compared to in vivo. In vitro models can be categorized into the properties the study is testing for: physicochemical and biological (Table 3).

Table 3. Illustrates the types of in vitro testing models

Physicochemical properties	Biological properties
1. Mechanical (stress , strain, maximal load) 2. Flexibility 3. Topography (spatial structure) 4. Degree of polymerization 5. Surface chemistry	1. Cytotoxicity 2. Genotoxicity 3. Enzymatic degradation 4. Cell proliferation 5. Cell adhesion 6. Length of the neurite growth 7. Cell density

Main vitro methods were testing

1. The physicochemical properties of the conduit include:
   i. Maximum load and breaking load using Instron Series IX Automated Materials Testing System.
   ii. Flexibility of the conduit was assessed by texture evaluation methods.
   iii. Spatial structure of the nerve guide conduit analyzed by scanning electron microscope (SEM).
   iv. The distribution of the microspheres analyzed by was light microscope (LMS).
   v. Degree of polymerization by Gel Electrophoresis.
   vi. Tensile strength and tensile strain: analyzed by scanning electron microscope (SEM) [3].

2. Biological properties of nerve conduit include:
   i. Cytotoxicity assessed by cell culturing and then electron microscopy coupled with immunocytochemistry [39] recommended by the ISO 10993-11.
   ii. Genotoxicity: by Ames test test [40] evaluates the mutagenicity in a bacterial reverse mutation system.
   iii. Enzymatic degradation: phage-born endosialidase [41].
   iv. Mass Spectrometric Analysis: using an Aquity UPLC. [41].
   v. The length of the neurite growth [42].
   vi. Cell proliferation and cell adhesions by fluorescence microscopy [41].
   vii. State of the nerve conduit combined with mesenchymal stem cells (MSCs) was assessed immunofluorescence [43].

I believe there are still elements of In-Vitro models that could be explored further to assist in development the optimal nerve conduit and in vitro nerve testing models are more easily more and standardized [44].

In conclusion, nerve regeneration with synthetic materials is challenging. The essential progression to human clinical use and trial had not been achieved despite 50 years of research. The reason may be multifactorial, including the complexity of nerves, limited understanding of neurophysiology, and the vast diversity of clinical nerve injury. Over 70 types of nerve conduit materials have been tested over the last few decades, clearly highlighting the unsatisfactory results produced by these synthetic conduits.

The lack of progression may be contributed by the limited consensus and consistency on functional testing methods for nerve conduits, in particular, in vivo models. In vitro testing models were often focused on preliminary testing for conduit toxicity and compatibility, rather than functional outcomes, and appeared to have some standardized method. The concept of in vitro functional testing had recently faded, and had shifted towards in vitro models. We believe there are still areas of in vitro nerve testing that can be expanded and applied for functional testing use. In vitro testing is safe, experiences less ethical dilemmas, and if testing models were able to mimic human environment, the use of in vitro studies may become superior to in vivo studies.

For in vivo studies, there are many reasons why they had not been a uniform methodology for testing nerve conduit. First of all, studies varied in their experimental goal, i.e., the type of nerve required for regeneration and the gap required for bridging. Secondly, resources and financial implications, which will influence the choice of animal model and testing equipment used. Therefore, it is impossible and impractical to have a universal method to suit all. However, some standardization should be discussed and formulated by the leading research groups in this field of tissue engineering. This will greatly facilitate the growth in this field, as a more consistent method will allow greater cross examination amongst studies.

Finally, we suggest more studies are to be conducted to cross-examine testing methods and animals used, as there is currently no literature to compare the quality of different methods. We believe that tissue engineering can still be the answer to nerve regeneration.

Researchers would greatly benefit from a unified methodology of in vivo testing and exploration into functional testing models for in vitro testing would also be beneficial. The emerging use of stem cell and growth factors into nerve conduits are showing some promising results, and we hope this may accelerate progress in this field.

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Assessment of Mitral Regurgitation Severity by Different Echocardiographic Methods in Comparison with Left Ventricle Angiography

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Abstract

Background Proximal isovelocity surface area method and mitral regurgitatant jet estimation (with its ratio to left atrium) are reliable methods for estimation of mitral regurgitation severity aiding in management strategy, precluding the use of other invasive procedures for assessment (e.g. left ventricular angiography).

Objective To study the usefulness of proximal isovelocity surface area and regurgitant jet size (with its ratio to left atrium) to determine the severity of mitral regurgitation in comparison with left ventricle angiography.

Methods Forty patients with mitral regurgitation planned to do left ventriculography to assess mitral regurgitation severity were studied. Estimation of mitral regurgitatant jet (and its ratio to left atrium) by color tracing of maximal jet area and estimation of effective regurgitant orifice, regurgitant volume and regurgitant fraction by proximal isovelocity surface area method were reported within 24-48 hours after angiography.

Results The effective regurgitant orifice, regurgitant volume and regurgitant fraction in correlation with angiographic grades were 46.67±27.mm^2 ($P < 0.0001$); 55.3 5±27.67 ml ($P < 0.0001$) and 57.41±20.63 % ($P < 0.0001$) respectively. The thresholds for severe mitral regurgitation (grade 4) were 60 mL, 52%, and 42.5 mm^2 for regurgitant volume, regurgitant fraction, and effective regurgitant orifice, respectively. Significant correlation ($P = 0.021$) of mean mitral regurgitant/left atrium ratio with the corresponding angiographic grade in central mitral regurgitation, while no significant correlation ($P = 0.799$) was found in eccentric mitral regurgitation.

Conclusion Proximal convergence method and estimation of mitral regurgitant size (ratio to left atrium) by color tracing of maximal jet area (in central jet) allows accurate estimation of the mitral regurgitation severity.

Keywords Proximal isovelocity surface area method, effective regurgitant orifice, mitral regurgitant volume

List of Abbreviation: MR = miral regurgitation, PW = pulsed wave, CW = contentious wave, LA = left atrium, LV = left ventricle, EROA= effective regurgitant orifice area, RVol = regurgitant volume, RF = regurgitant fraction, PISA = proximal isovelocity surface area, VTI = velocity time integral, SV = stroke volume, CSA = cross-sectional area, LVOT = Left ventricular outflow tract, RAO = right anterior oblique, MVP = mitral valve prolaps, MV= mitral valve, A = area, V = volume, RAO = right anterior oblique, ROC = receiver operator curve.

Introduction

Mitral valve regurgitation has long been recognized as an important cause of morbidity and mortality, where those with severe regurgitation observed clinically experience excess mortality and high morbidity [(1)]. Consequently, determining the degree of regurgitation by doppler echocardiography is a crucial part of the clinical evaluation of patients with mitral regurgitation [(2,3)].

To define the degree of regurgitation quantitatively, new methods and new concepts using Doppler echocardiography have allowed the measurement of regurgitant volume (RVol),

regurgitant fraction (RF) [4,5,6] and effective regurgitant orifice (ERO), a measure of lesion severity [6,7].

Proximal isovelocity surface area (PISA) measurement is used in echocardiography to estimate the area of an orifice through which blood flows. The PISA method is based on 1) the properties of flow dynamics and 2) the continuity principle [8].

When a liquid (in this case blood) is forced from a large chamber into an orifice at a constant flow, its particles accelerate towards the orifice until the velocity is greatest at the narrowest point of the orifice. This acceleration occurs along a series of concentric "hemispheres" or "hemishells" whose center is at the orifice itself. Those hemispheres are contained in an area referred to as the flow convergence area [8] as shown in figure 1.

The volume of a liquid going through a conduit per unit time, called the flow rate, equals the cross-sectional area of that conduit times the velocity of the liquid: Flow rate = area × velocity. Liquids, by definition, are essentially incompressible. Therefore, the continuity principle dictates that in the absence of a leak in the conduit or additional input, the flow rate is constant along the length of the conduit [9]. If the cross-sectional area decreases, the velocity must increase to compensate and *vice versa*. The same holds true for the heart and, assuming there is no shunt, the flow rate throughout the heart is constant. What changes is the area and velocity of the column of blood as it flows through the various parts of the heart. As the area changes, the velocity of the blood must change also, according to the continuity equation:

$A_1 \times V_1 = A_2 \times V_2$ where A = area, V = velocity [8].

The first step in the PISA method is to demonstrate the mitral regurgitant (MR) jet by color flow Doppler and to calculate the flow of blood within the flow convergence area. The flow convergence area is the colored area on the ventricular side of the mitral valve in systole. This area contains an infinite number of concentric hemispheres along which the blood accelerates towards the regurgitant orifice [8].

By Doppler convention, the MR jet is displayed in shades of red, as blood cells accelerate, the color goes from dark red to bright red, to orange, to yellow. When the cells reach the aliasing velocity (also known as the Nyquist limit), the color suddenly changes to blue. This is the point of interest, at which the velocity is known with certainty. (The Nyquist limit is defined as the velocity at which the color flow switches from red to blue or blue to red.) Radius is measured from the orifice to point of colour change. If the flow convergence is not a true hemisphere, the angle subtended by the flow convergence at the orifice has to be measured and divided by 180 to get a correction factor. The next step consists of measuring the maximum velocity of blood at the mitral regurgitant orifice using continuous wave (CW) Doppler of the MR jet. As usual, one must make sure that the Doppler beam is lined-up with the MR jet

Finally, one calculates the EROA using the initial formula [8]:

$A_1 \times V_1 = A_2 \times V_2$

$EROA \times V_{max\,(CW)} = 2\pi r^2 \times$ Nyquist Limit

$$EROA = \frac{2\pi r^2 \times \text{Nyquist Limit}}{V_{max\,(CW)}} \times \frac{\alpha}{180}$$

Putting in mind that (α) is the angle correction factor; (r) is the radius of the hemispheric flow convergence. Knowing that Volume = area × VTI, one can trace the velocity-time integral (VTI) of the MR jet on the CW signal, and one can calculate the mitral RV (RV_{MR}), using the following equation [8]:

$RV_{MR} = EROA \times VTI_{MR}$

Once the RV is known, one can calculate the ratio of RV over total stroke volume, a value known as mitral regurgitant fraction [8].

RF=RVol/ (regurgitant vol+aortic stroke volume).

Aortic SV = CSA (at LVOT) × VTI = $\pi d^2/4 \times VTI = 0.785d^2 \times VTI$ [8]. .

The area of the regurgitant jet relative to the size of the left atrium (LA) has been shown to

correlate well with regurgitant severity determined with angiography when the jet is not eccentric where the flow jet is directed against the atrial wall and appears smaller than a free jet of the same regurgitant volume (Coanda effect) [10]. It was estimated that MR/LA ratio best correlating to angiographic grade is as follows [11]: <20% = mild, between 20-40% = moderate and >40% = severe.

The goal of the our study is to assess the validity of proximal isovelocity surface area method (PISA) and MR size (with its ratio to LA) as measures to determine the severity of mitral regurgitation instead of invasive methods.

Methods

A prospective study was conducted at Ibn Al-Bitar and Al-Nassirya Cardiac Centers from May 2011 to May 2012 on 40 patients known to have MR planned to do LV ventriculography to assess MR severity for possible subsequent surgical referral. Patients were subjected to a complete echocardiographic assessment within 24-48 hr's after angiography with the aid of an expert echocardiographer, and then comparative analysis of data collected from each patient was performed.

Angiographic Assessment

In each patient, left ventriculography was performed in the 30° right anterior oblique (RAO) projection with 40-50 ml of iohexol iodine contrast injected over 2-3 seconds at 15-20 mL/sec, 600 pounds per square inch (PSI) (a pressure stress unit). The angiographic severity of mitral regurgitation was graded according to a historically accepted grading scheme (Seller) [12] (i.e. from 1-4).

Echocardiographic Analysis

A comprehensive Doppler echocardiographic examination was performed and analyzed as described previously, echocardiography machine used was Philips (Invisor C), and aliasing velocity range was between 40-64 cm/sec.

The mechanism of mitral regurgitation was determined on the basis of the two-dimensional appearance of the left ventricle, subvalvular apparatus, and valve leaflets and the dimension of the mitral annulus. Organic mitral regurgitation was characterized by intrinsic valvular disease, and ischemic/functional mitral regurgitation was characterized by normal valves, enlarged annulus, and global or regional left ventricular dysfunction. Assessment of mitral regurgitation was performed by two methods:-

1- Measuring (ERO, RVol and RF) through proximal isovelocity area method using the 4-chamber view through apical axis with applying angle correction factor if the flow convergence hemisphere is restricted laterally by the mitral leaflet or ventricular wall.

2- Measuring the ratio of MR/LA size. The jet area was measured as the largest clearly definable flow disturbance in the apical view, and expressed as the maximal jet area. Beside that, any additional valvular lesions were described.

These data were used to classify MR from the severity point of view to mild, moderate, moderate to severe and severe as shown in table 1 [13,14]:

RVol = regurgitant volume, RF = regurgitant fraction, ERO = effective regurgitant orifice are

Table 1. Classification of Mital Regugitant

Grade	RVol (mL)	RF %	EROA, mm^2
1	<30	<30	<20
2	30-44	30-39	20-29
3	45-59	40-49	30-39
4	≥60	≥50	≥40

RVol = regurgitant volume, RF = regurgitant fraction, EROA= effective regurgitant orifice area.

Patients

Patients included had pure chronic MR and were subjected to left ventricular angiography and quantitative Doppler echocardiography for quantification of mitral regurgitation severity by both PISA method and MR/LA size 24-48 hr between each other. Patients who were excluded from the study had associated aortic valve disease or mitral stenosis; had change in systolic blood pressure ≥30 mmHg between

(angiography and echo studies); had accidental discovery of MR by angiography (due to improper injection preparation) and who had multiple regurgitant jets by color Doppler.

Statistical analysis

Data were analyzed using SPSS version 16 and Microsoft Office Excel 2007. Numeric data were expressed as mean ± SD. Nominal data were expressed as number and percent. ANOVA test was used to compare means among groups. The candidate thresholds that were considered to best separate the continuous quantitative variables in correspondence with the angiographic grades were those with the highest sum of sensitivity and specificity and the lowest value of their difference. Chi-square test was used to study association among nominal variables. Receiver Operator Curve (ROC) was used to identify cut off values with their corresponding sensitivity and specificity. P-value less than 0.05 were considered significant.

Results

The 40 patients (21 men and 19 women) included were (20-82) years old (mean 56.18±2.06). Distribution of age showed most of age groups were in 6th and 7th decade. The mechanism of mitral regurgitation was ischemic / functional (n=15) or organic (n=25), in both genders MVP followed by ischemic cause were more prevalent. 70% (n=28) were in sinus rhythm, 30% (n=12) in atrial fibrillation. Systolic blood pressure was 118.12±15.91 mmHg at echocardiography and 120.50±16.74 mmHg at angiography (P = 0.518). The mitral regurgitation grade by angiography was 12.5% for grade 2, 32.5% for grade 3, 55% for grade 4.

The distribution of patients among the four angiographic grades and the values of RVol, RF, and ERO for each angiographic grade are summarized in the following figures (2-4) as there were notable overlaps between grades**,** as expected, but despite this, the differences between each angiographic grade in terms of RVol, RF, and ERO were significant (overall P < 0.001, P < 0.001, and P < 0.001 for RVol, RF, and ERO, respectively) as shown in table 2.

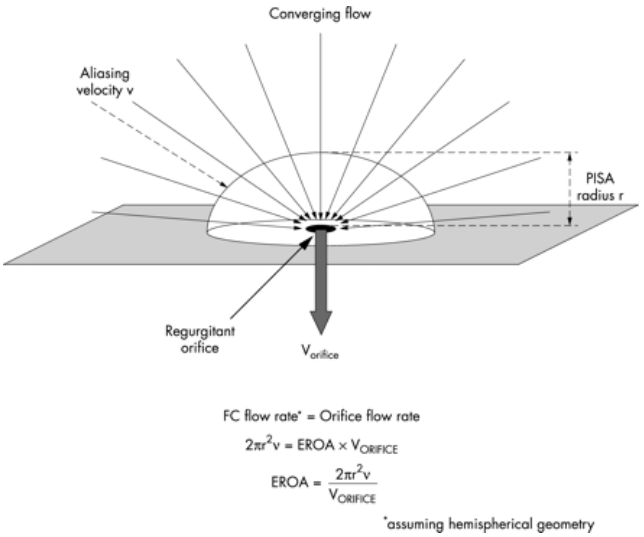

Fig.1 Diagram showing converging flow area of mitral regurgitation

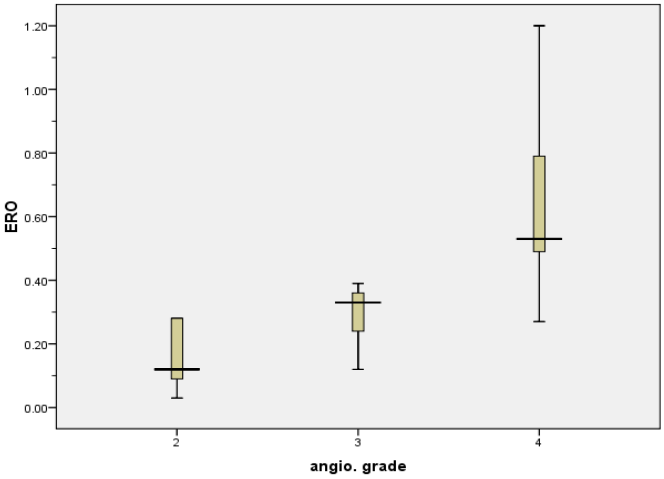

Fig. 2. Box plot of mean effective regurgitant orifice area for each angiographic grade

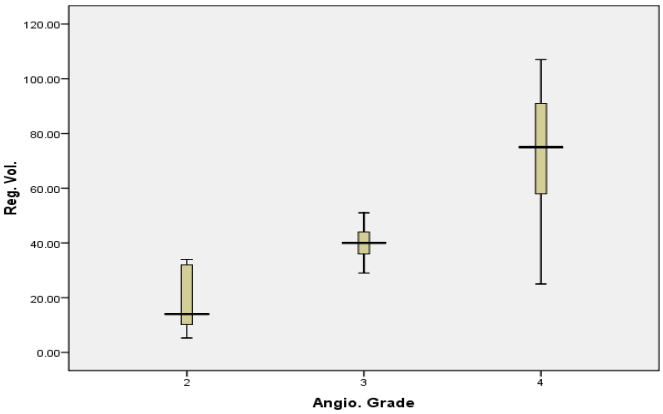

Fig. 3. Box plot of mean Regurgitant volume for each angiographic grade

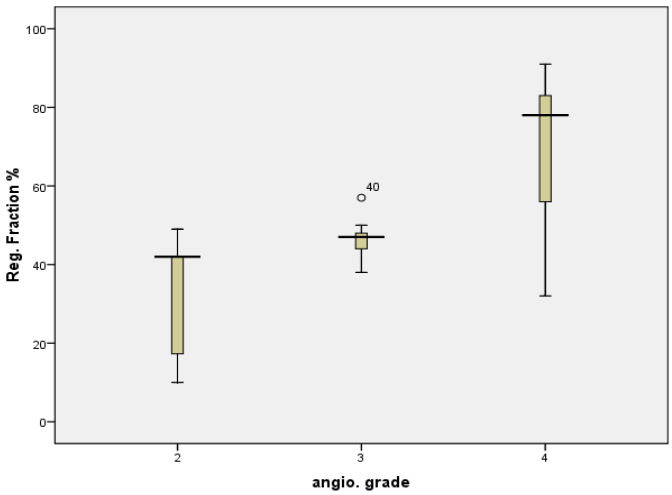

Fig. 4. Box plot of mean regurgitant fraction for each angiographic grade

Threshold values for ERO, RVol, and RF corresponding to each angiographic grade are listed in the table 3, with the descriptors of their diagnostic values. The thresholds that were finally selected as best separating the grades with the best sensitivity and specificity results. Regarding eccentric lesions, about 63% (n=25) were eccentric, 37% (n=15) were central MR, mechanism of MR in eccentric lesions correlated with MVP more than central lesions ($P < 0.05$).

In comparing eccentric and central MR regarding correlation of angiographic and echo-cardiography parameters, it showed no significant difference between them, although about 3 individual cases of eccentric MR who had grade 4 by angiography showed limited correlation with quantitative echocardiographic parameters (ERO, RV, RF) i.e., underestimation of real measures.

The minimum radius (of the hemispheric flow convergence) diameter on echo estimation that correlates best with grade 4 (severe) MR in both central and eccentric MR were eccentric 0.55 cm and central 0.83 cm.

Regarding MR jet and ratio to LA size (MR/LA) ratio in studied patients, it fell in two groups those with central jets and those with eccentric jets. The central MR; significant correlation ($P = 0.021$) of mean MR/LA ratio with the corresponding angiographic grade as a whole, especially in the severe grade (grade 4) as shown in figure 5.

The Eccentric MR; no significant correlation ($P = 0.799$) in total between the mean of MR/LA ratio to the angiographic grade especially in the severe grade (grade 4) as shown in figure 5. The least MR size that correlates with grade 4 in all patients was 9.6 cm^2 (sensitivity 72.7%, specificity 88.9%. The least MR size that correlates with grade 4 in patients with central MR was also around 9.6 cm^2 (sensitivity 85.7%, specificity 87.5%) i.e., comparable specificity and more sensitivity.

Regarding the correlation of MR area with ERO, Reg. volume and Reg. Fraction, it showed significant correlation with ERO, but not with other parameters ($P \leq 0.05$).

Table 1. Quantitative variables corresponding to each angiographic grade

Angiographic grade	1	2	3	4	*P* value
Number	40	**5**	**13**	**22**	
ERO mm^2	46.67±27.33	16.00±11.42	30.38±8.06	63.27±25.47	<0.001
RVol ml	55.35±27.67	19.10±13.07	40.00±6.30	72.66±24.37	<0.001
RF %	57.41±20.63	32.06±17.24	46.38±4.62	69.68±18.33	<0.001

ERO = Effective regurgitant orifice, RVol = Regurgitant volume, RF = Regurgitant fraction

Discussion

From the results one can see that the commonest cause of MR in both sexes was MVP (myxomayous MV), probably because of the small number of patients and because of our exclusion criteria of patients selection, as we excluded any MV regurgitation with coexistent mitral stenosis and aortic valve disease (i.e. we excluded many rheumatic causes).

Table 2. Diagnostic value of the thresholds of Doppler quantitative variables corresponding to the angiographic mitral regurgitation grades

Parameter			Sensitivity	Specificity
Effective regurgitant orifice	Grade 2 versus 3	29 mm^2	69.2%	100%
	Grade 2 and 3 versus 4	42.5 mm^2	81.8%	100%
Regurgitant volume	Grade 2 versus 3	34.5 ml	84.6%	100%
	Grade 2 and 3 versus 4	60 ml	72.7%	100%
Regurgitant fraction	Grade 2 versus 3	43%	84.6%	80%
	Grade 2 and 3 versus 4	52%	81.8%	94.6%

Hemodynamic conditions (like systolic hypertension, LV contractility) should be standardized at echo and angiography and no significant difference (as in our study) should exist as any change in loading conditions between echocardiography and angiography could account for some of the misclassifications between quantification and angiographic grade [15].

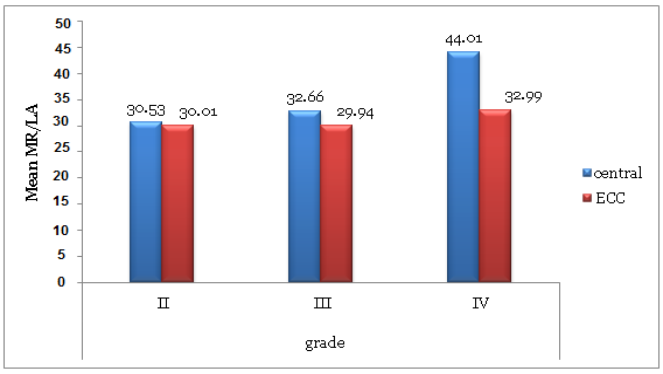

Fig. 5. Difference between central and eccentric MR regarding correlation between MR size and angiographic severity

Because most patients in our study originally had presumed moderate-severe MR and were sent for cath. Before possible surgery, grade 4 followed by grade 3 MR was found most of the time followed by grade 2. Grade 1 was not encountered as they were not sent for angiography from the beginning (regarded as mild), those MR found on angiography accidentally was not included because of the miss-preparation of injection parameters.

Quantitative methods allow the measurement of RVol and RF to assess volume overload and the measurement of ERO area, a surrogate of lesion severity [7]. All angiographic grades showed significant differences in RVol, RF, and ERO, demonstrating that a statistical (in addition to visual) separation between grades is present and this is comparable to results achieved by Dujardin and colleagues [14] and in other previous studies [5,16] who showed a similar significant correlation of quantitative echo parameters in regard to angiographic grade. Table 2 reveals the cut off values of ERO, RV, RF separating each grade with best sensitivity and specificity obtained and as far we are concerned more with advanced degrees of MR we took the best cut-off values earned in the study that separates values equivalent to grade 4 from other grades, i.e., the best ERO value was 42.5 mm^2 (Sensitivity 81.8%, Specificity 100%), RVol. 60 mL (Sensitivity 72.7%, Specificity 100%), RF 52 % (Sensitivity 81.8%, Specificity 94.6%), which is nearly comparable to cutoff thresholds in other studies [14] and references [13].

If there are multiple regurgitant orifices, the flow convergence method may be completely inaccurate in estimating the EROA [8], so we excluded any patient with multiple jets MR from the study.

Angle correction factor α applied when the hemisphere of the flow convergence is limited by valve leaflet or ventricular wall (i.e., If the base of the hemisphere is not a flat surface) to make accurate results [17], but still it can be a limiting factor in applying PISA in quantitative measures as measurement of angle factor is not available in our echocardiography device software and needs off-line analysis and measurement by the operator. As color Doppler

parameters are adjusted, the hemispheres may become more flattened or more cone-shaped, even if the regurgitant orifice is circular. Again, the equation $2\pi r^2$ may not apply. It has been reported that the PISA hemispheres are closest to being true hemispheres when their radius optimized by adjusting the Nyquist limit [18], which in most echocardiography machines is done by turning a knob identified as "pulse repetition frequency" or "color Doppler scale."

Regarding analysis of MR/LA size ratio data, fitness between MR/LA size ratio and angiographic grade goes more with central lesions than eccentric one, i.e., for same grade 4 mean ratio of MR size reaches 44% in central MR while it's 32% for eccentric MR (theoretically should be more than 40%), i.e., there is underestimation and this goes with the fact that eccentric, wall-impinging jets appear significantly smaller than centrally directed jets of similar hemodynamic severity, mainly because they flatten out on the wall of the receiving chamber [19]. Also Enriquez-Sarano and associates [20] stated that mitral regurgitant jet eccentricity influence jet extent and the same regurgitant volume produces smaller jet areas for eccentric compared with central jets [18,19].

In our study, minimal MR area found to correlate well with severe angiographic grade was 9.6 cm^2 (as a total including central and eccentric MR) with sensitivity of 72.7% and specificity of 88.9% beside a sensitivity of 85.7% and specificity of 87.5% (if only central MR was involved). This was comparable to a study conducted by Spain and coworkers [21] who revealed that maximal jet area greater than 8 cm^2 predicted severe mitral regurgitation with a sensitivity of 82% and specificity of 94%.

We can see good correlation between maximal jet size and ERO (P = 0.042) but weak and non-significant correlation with RVol and RF (P = 0.133, P = 0.863, respectively). Our result agrees with what Enriquez-Sarano and colleagues [20] has found that weak correlations exist between regurgitant volume and jet area and regurgitant fraction and jet area/left atrial area ratio, also Spain and colleagues [21] showed that jet area measurements have limited correlation with regurgitant volume and fraction.

In conclusion proximal convergence method (PISA) and MR/LA ratio (in central jet) allows accurate estimation of the mitral regurgitation severity and correlates well with MR severity estimation by LV angiography.

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Declaration of interest

The author declare no conflict of interest

Author contributions

Dr Rafid did the echo and angiography studies and most of the statistical and fine touches of the study and Dr Muthanna collect the cases.

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Safety and Efficacy of Single-Session Nonstented Laser Ureteroscopic Lithotripsy

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Abstract

Background Ureteric stone is quite common; management involve medical and surgical intervention. Surgically the uretroscope is the most commonly used instrument.

Objectives This study was done to demonstrate the safety and efficacy to fragment and remove ureteral calculi with an ureteroscope using Holmium laser and render patients stone free with a single procedure without the need for ureteral stenting.

Methods One hundred and twelve patients aged between 4 to 63 years, who proved to have ureteric stones regardless the size or location of those stones, where subjected to ureteroscopy procedure under spinal or general anesthesia, and Holmium laser was used to fragment the stones.

Results The majority (92.86%) of those patients was stone free with single session, no stent was left.

Conclusion The stone can be disintegrated and achieving stone free state in single session and no stent was needed.

Key words Laser, Holmium, ureteroscope, stent, stone

List of abbreviations: ESWL = Extracorporeal shock wave lithotripsy, EU = Excretory urogram, CT= Computed tomography, URS = Ureteroscope, F = French, nm = nanometer, KUB = Scout plain x-ray (kidney, ureter, bladder).

Introduction

The goal of the surgical treatment of patients suffering from ureteral calculi is to achieve complete stone clearance with minimal attendant morbidity.

Improvements in surgical technology, such as extracorporeal shockwave lithotripsy (ESWL), rigid and flexible ureteroscopes, the holmium laser, and basket devices, have greatly augmented the urologist's ability to efficiently treat such patients, regardless of the size or location [(1)].

Ureteric stone is quite common; management involve medical and surgical intervention. Surgically the uretroscope is the most commonly used instrument, intracorporial lithotripsy used mostly ultrasonic, pneumatic and laser lithotripter. Holmium laser is the best for lithotripsy its safety and efficacy make it superior to the other [(2,3)].

The ability of the holmium laser to fragment all stones regardless of composition is a clear advantage over other modalities [(1)].

Holmium laser is one of the safest, most effective, and most versatile intra-corporeal lithotripsy anywhere in the urinary tract [(4)].

This study was done to demonstrate the safety and efficacy to fragment and remove ureteral calculi with an ureteroscope using Holimum laser and render patients stone free with a single procedure without the need for ureteral stenting.

Methods

From Nov. 2011 till Dec. 2012, 112 patients, aged from 4 years to 63 years (average 36 years), having ureteric stones, proved by ultrasonography, scout plain x-ray (KUB), excretory urography (EU) or computed tomography (CT) scan were admitted to the Surgical Specialty Hospital and Nursing Home Hospital, Medical City Complex.

Ureteroscopy was done using 7 F and 8.5 F semirigid ureterscope (according to the availability), the ureteric orifice was dilated using ureteral dilators in some patients, and the uretreoscope was advanced carefully to the stone sites over hydrophilic guide wires. Holmium laser with low power and low frequency setting was delivered through 350-600 nm laser fiber; stones were carefully distracted to small fragments not more than 3 mm in size, stone retrieval with basket for larger fragments used infrequently. Ureteral stenting was unnecessary in majority of the cases.

Those patients where followed within 14 days with ultrasonography, KUB, urinalysis to prove stone free-state.

Results

Stone sizes ranged from 6 mm to 20 mm (average 11 mm), localized as 38% at the lower ureter, 45 % at the mid part of the ureter and 17 % at the upper portion of the ureter.

Total disintegration of stones with fragments less than 3 mm; was achieved in 104 patients and no any kind of stent was left behind. Ureteric stents (JJ stent) were used only in 5 cases where big stone fragments pushed back to the kidney or ureteric perforation occurred. The demographic criteria of the patients were shown inn table 1.

Among the total number of cases, three of them (2 impacted pelviureteric junction stones, 1 mid ureteric stone) were unsuccessful, stones were so impacted and ureters were kinked and un-negotiable by ureteroscope, then they were converted to open surgery.

Perforation occurred twice, treated with JJ stent, (< 2%) as seen in figs. 1 and 2.

Table 1. Demographic features of patients who had ureteroscopy with laser.

Feature	No. (Average)
No. of patients	112
Sex ratio	69 M/43 F
Age range	4-63 year
Stone size (range)	6-20 mm
Success rate (non-stented)	104 (92.86%)
Failure rate	8 (7.14%)
Stented rate	5 (4.46%)

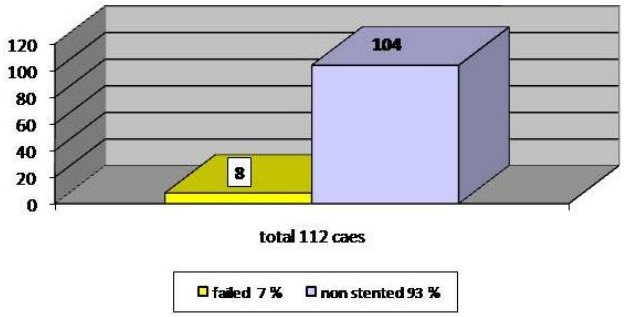

Fig. 1. Distribution of results successful versus failed cases.

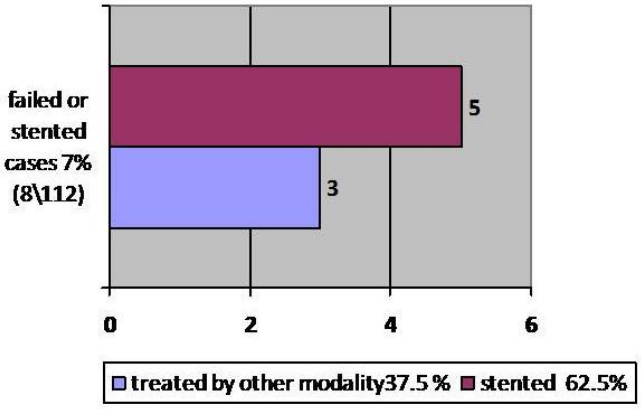

Fig. 2. Distribution of failed cases (stented, treated by other modalities)

Discussion

Ureteroscopy is the most effective way for ureteric stone treatment regardless its location or consistency [1,5,6], rate of disintegration reach 92% usually in single session [7]. In the present study 93.75% full disintegration of the stones was achieved in single session with aid of repulsion prevention instruments [8]. Stone retropulsion can result in increased operative time and cost-resulting from the need to change

from the semi-rigid ureteroscope to a flexible instrument to chase migrated calculi, additional procedures to treat residual migrated fragments are often required(9). In our study, it was 2.33 % compared to 3.3% in another study [10]. Careful negotiation, low power laser and dual channel ureteroscope that we used can lower the risk of retropulsion.

Stenting of the ureters after stone disintegration varies, gravels may be retrieved with baskets if they are big or they pass spontaneously [11]. We use stent only in limited cases, (5\112) in another study (2/53) [10]. Avoiding stents lowers costs and gives fewer irritative symptoms.

Stentless ureteroscopic holmium laser disintegration without gravels removal is a safe and effective method for pediatric ureteral stone less than 10 mm in diameter [12].

Uncomplicated ureteroscopic lithotripsy can be safely performed without the placement of a ureteral stent. Patients without stents had less operative time, pain and hematuria [13].

In the present study ureteric stented only in 5 cases 2 for ureteric injury and 3 for adjuvant ESWL because of big gravels push back to the kidney. No significant complications and the patient were kept on tamsolusine 0.4 microgram once per day and anti-inflammatory (diclofenac sodium) 50 mg for 7-10 days to facilitated gravel passage together with encouraged fluid intake.

Ureteroscopic laser lithotripsy is recommended as the treatment of choice for distal ureteric calculi in children; we had 12 child treated with the small ureteroscope (7F) with laser all of them had distal ureteric stone and all were stone free. Using small ureteroscopes the target stone was treated safely and effectively [6,14,15].

In conclusion, treatment of urteric stone with ureteroscope and holmium laser have a high success rate with very low complications. Although ureteric stenting is only indicated in limited cases, stent less ureteroscopic holmium laser disintegration without gravels removal is a safe and effective. The procedure is safe with high success and promising in treatment even in pediatric urterolithiasis.

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Author contribution

Patients were referred from the clinics and surgical subspecialty's outpatient of all authors and ureteroscopies were done by all authors almost equally, the theoretical and statistical parts were divided to be done almost equally by all authors.

Conflict of interest

There are no any financial and personal relationships that could bias this work.

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Ocular Abnormalities among Deaf Students in Aden City, Yemen

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Abstract

Background The incidence of ophthalmologic abnormalities among the deaf children is high, compared with the hearing population of the same age.

Objective The main objective of this study was to determine the ophthalmologic abnormalities among the students attending deaf and dumb's school in Aden city (Yemen).

Methods This was a cross sectional descriptive study carried out from October 2012 to March 2013 in a school for the deaf and dumb in Aden (Yemen). All students who consented to participate in the study, were enrolled and subjected to a detailed ophthalmic examination, including visual acuity (VA), ocular motility, slit-lamp and fundus examination conducted by a qualified ophthalmologists.

Results: A total of 138 deaf students (90 males, 48 females with a male to female ratio of (2:1) were included in this study. A high proportion of the students (92; 66.6%) were in the age group >14 years. Normal eye examination was found in (51; 36.9%), ocular abnormalities found in (87; 63.1%), with some students having multiple abnormalities. Refractive errors comprised the leading abnormality in 41.3%, retinal pigment epithelium patches was found in 10.9% and Warrdenburg syndrome in 5.8%. Twelve eyes 4.4% exhibited VA < 6/18).

Conclusion Ocular abnormalities in deaf students are remarkably high which indicate the importance of early ophthalmologic examination in order to facilitate adequate integration of the deaf student as a useful and productive member in the society.

Key words Deafness, ocular abnormalities, refractory errors; visual impairment, Yemen.

List of abbreviation: VA = Visual acuity, D = Diopter, ERG = Electroretinogram.

Introduction

Ocular problems are more common in children with hearing problems than in normal children. The high prevalence of ocular abnormality in deaf children may be attributed to important elements of the eye and ear (example retina and cochlea) maturing during the same embryological stage, from the same embryological layer, which may be susceptible to genetic or environmental factors such as hypoxia, toxic agents, viruses meningitis and other conditions, which may affect both eye and ear [1-3].

Neglected visual impairment could aggravate the educational and social disability so ophthalmologic screening and detection of visual problems in deaf children is important. The vast majority of knowledge is obtained through the sense of sight and hearing, some through the tactile, kinesthetic and olfactory sense. When one of these senses is seriously impaired, the other is used to compensate the disability, and as the degree of impairment increases the role of the remaining sense becomes progressively more significant. So the deaf population may

compensate by making great use of visual perceptual cues than their hearing peers. Thus, even a mild refractive error may reduce the visual cues available to the deaf mute person [4].

Many researchers reported a high incidence of ophthalmologic abnormalities among the deaf children compared with the hearing population of the same age [5-7]. A review of the literature suggested 35 to 57% visual defects among hearing impaired children compared with 17 to 30% among normal hearing children [1,6,8]. Hence, particular attention must be paid to ocular abnormalities in deaf children as they may be correctable (as myopia) or treatable (as cataract). Prompt identification is of utmost importance to optimize language development (spoken, sign or both) and develop social cognition. Children with non-correctable and non treatable visual disorders, such as retinitis pigmentosa in Usher's syndrome require multiple environmental adaptation and appropriate support services.

The main objective of this study was to determine the ophthalmologic abnormalities in students attending deaf school at Aden city, Yemen and to provide treatment for those with remediable conditions.

Methods

This was a cross sectional descriptive study conducted from October 2012 to March 2013 at Alnoor School for the deaf and dumb in Aden city (South of Yemen). Ethical clearance for the study was obtained from the Research and Ethics Committee of the Faculty of Medicine and Health Sciences, University of Aden.

A total number of 138 hearing impaired students (irrespective of the degree of hearing impairment) were examined with the presence of a schoolteacher. The children responded by sign language which was interpreted by the teacher or by sign and oral communication when possible. The required information was derived from either parents or teacher.

The ophthalmologic examination included visual acuity (VA) assessment by using a Snellen's or tumbling E chart at 6 meters (cycloplegic refraction was done on those students who were 6-10 years of age). Pupillary evaluation, ocular motility examination, alternate cover test, anterior segment examination by slit lamp biomicroscopy was conducted. Fundus examination was done by qualified ophthalmologists using a direct ophthalmoscope preceded by 1% tropicamide for pupillary dilatation. Intra-ocular pressure was recorded when necessary. Myopia was defined as an error more than or equal $\geq$ to -0.5 diopter (D), hypermetropia as $\geq$ +1 D, and astigmatism as $\geq$ -0.5 D, anisometropia as difference in refraction between the two eyes $\geq$ 2.0 D and amblyopia was defined as best corrected VA of less than 3 in either eye resulting from anisometropia, strabismus or large astigmatic error. Extra ocular muscle imbalance was noted when eye misalignment was $>$ 5 degrees. Electrodiagnostic tests were not performed. Medicines were administered to those with treatable eye diseases and glasses were provided for those who needed them.

Statistical analysis was conducted by the SPSS program (SPSS 18). The data were parametric in distribution and tested by the Chi square test and the student t-test for the difference between 2 means. The tests were conducted with the 95% confidence interval and a *P*-value of $\leq$ 0.05 was considered statistically significant.

Results

A total of 138 deaf students were enrolled in this study. They were 90 (65.2%) male and 48 (34.8%) female students, giving a male to female ratio of 2:1. The age range was between 3-23 years, mean age 16.5 ± 5.3 years. A high proportion of the students were in the age group $>$ 14 years 92 (66.6%) (Table 1).

Statistically, there is no significant difference between the mean age of male compared to female deaf students ($P > 0.05$) and the Chi-square did not show any significant association between the age groups of deaf students in both sexes ($P > 0.05$) (Table 1).

Table 1. Age group and gender distribution of the studied deaf students

Age group (years)	Male		Female		Total	
	No.	%	No.	%	No.	%
3-6	3	2.2	3	2.2	6	4.4
7-10	10	7.2	6	4.4	16	11.6
11-14	21	15.2	3	2.2	24	17.4
15-18	20	14.5	12	8.6	32	23.1
> 18	36	26.1	24	17.4	60	43.5
Total	90	65.2	48	34.8	138	100.0
Mean ± D* (Range)	16.3 ± 5.1 (3 – 23)		17.0 ± 5.7 (3 – 23)		16.5 ± 5.3 (3 – 23)	

Percentages calculated from the total sample size (n=138), Chi square test [χ^2: 6.7, *P*: 0.15] is statistically insignificant, * T-test for the difference between 2 means [F: 0.598, *P*: 0.441] is statistically insignificant

Normal eye examination was detected in 51 (36.9%) while 87 (63.1%) showed ocular abnormality with some having multiple ophthalmologic abnormalities. Table 2 shows the major ocular abnormalities detected in those students, refractive errors comprised the leading ocular abnormalities in 41.3% of the students. Astigmatism was found in 21.7%, Hypermytropia in 10.9% while myopia was found in 8.7%. Around 15 students 10.9% showed patches of retinal pigment epithelium, which presumed the existence of Usher's syndrome. Anterior segment examination in 8 (5.8%) students revealed blue iris with white forelock which may be due to Waardenburg syndrome. Allergic conjunctivitis was found in 7 (5.1%), while corneal abnormality (keratoconus, microcornea) and maculopathy was detected in 6 (4.3%) of the students respectively for each anomaly, squint was found in 3 (2.2%) of deaf students.

Table 2. Results of ophthalmic examination in the studied deaf patients

Ophthalmologic findings		No = 138	
		No	%
Refractory errors	Hypermetropia	15	10.9
	Myopia	12	8.7
	Astigmatism	30	21.7
Retinitis pigmentosa (presumed Usher syndrome)		15	10.9
Iris (blue iris, white forelock hair presumed Woodenburg syndrome)		8	5.8
Allergic conjunctivitis		7	5.1
Cornea	Keratoconus	3	2.2
	Microcornea	3	2.2
Maculopathy		6	4.3
Squint		3	2.2
Normal findings		51	36.9

Percentages calculated from the total sample size (n=138), some patients had multiple ophthalmologic abnormalities at the same time.

A total of 252 eyes (91.3%) had normal vision with VA 6/6 – 6/18. Twelve eyes (4.4%) of the total eyes (n = 276) exhibited VA < 6/18 with 9 visual impairment and 3 severe visual impairment according to WHO classification. None of the patients were totally blind (Table 3).

Table 3: Visual impairment in deaf students based on WHO categorization

Visual acuity	No. of eyes	%	WHO category
6/6 – 6/18	252	91.3	Normal vision
< 6/18 – 6/60	9	3.3	Visual impairment
< 6/60 – 3/60	3	1.1	Sever visual impairment
< 3/60 NPL	-	-	Blind
Undeterminable	12	4.3	-
Total	276	100.0	-

Percentages calculated from the total number of examined eyes (n=276)

Table 4 compares the prevalence of ocular abnormalities and the specific different ocular findings in hearing impaired students in this study with similar reports in the literature.

Table 4. Studies in the literature investigating the prevalence of ocular abnormalities in hearing impaired students

Study	Year	Country	No.	Prev.	Findings
Ma et al [13]	1989	China	279	35.8%	17.9% refractory errors 29.3% retinal abnormalities
Elango et al [12]	1994	Malaysia	165	57.6%	14% refractory errors, 5% squint, 35% retinal abnormalities
Brinks et al [27]	2001	USA	231	48%	16% refractory errors, 21% retinal pig., 9% ocular hypertension
Hanioglu-Kargi et al [7]	2003	Turkey	104	40.4%	29.8% refractory errors, 18.2% squint, 8.6% retinal abnormalities
Guy et al [6]	2003	UK	110	43.6%	39.1% refractory errors, 6% squint, 11% retinal abnormalities
Al-Abdulgawad et al [11]	2005	KSA	302	61%	48.7% refractory errors
Parikshti et al [15]	2009	India	901	24%	18.5% refractory errors, 1.3% squint, 1.7% retinal abnormalities
Khanderkar et al [18]	2009	Oman	223		19.3% refractory errors
Abah et al [20]	2011	Nigeria	620	20.9%	7.9% refractory errors, 3.4% all. conj.
This study	2013	Yemen	138	63.1%	41% refractory errors, 2.2% squint, 15% retinal abnormalities

Prev. = Prevalence, pig. = pigment, all. conj. = allergic conjunctivitis

Discussion

Deaf children are known to be at increased risk for delayed language, speech, cognitive and social development [9,10]. Vision plays a key role in gathering information from the environment similar to the hearing process. This fact emphasizes the importance of ensuring that visual function is optimized in deaf children especially in the first few years of life during which there are many key developmental milestones. The great importance of hearing and vision is one of the reasons to advocate regular ophthalmic evaluation in deaf children.

In Yemen, several studies evaluated the overall prevalence of ophthalmological abnormalities in the general school population. To the best of my knowledge, this is the first report that describes the deaf school's students. In this study the prevalence of ocular abnormalities (63%) was high comparable to the reports in Kingdom of Saudi Arabia (KSA) (61%) and Malaysia (57.6%) [11,12], but relatively higher than in Turkey (40.4%) [7], China (35.8%) [13], Iraq (32%) [14] and India (24%) [15] . The variability of the results may be attributed to the differences in the age of patients, study population and the site of study (clinic versus institution). Table 4 compares the findings of this study with other similar studies in published literature.

All students with hearing loss are only admitted in one school for the deaf in Aden. It is quite possible that girls are not allowed (by families) to attend this school which explains the higher predominance of male (65.2%) over female (34.8%). This gender finding is similar to a study done in India (55%) male [16], but in contrast to a study done in Nigeria where female had a higher percentage (60.5%) [17]. It was recorded that a higher percentage of students were in the age group >14 years (66.6%) which may be due to the reason that no routine ENT examination is performed for the children in the early life in order to detect the deaf child and to evaluate his visual status.

Refractive errors were the most frequently encountered ocular abnormality in this study (41.3%) which is consistent with the findings of previous studies, 62.5% in Iraq,[14] (52%) in India,[16] (48.7%) in KSA [11], and (39.1%) in UK [6]. In other reports, Refractive errors were the commonest ocular abnormality but less frequent than our results, Turkey (29.8%) [1], Oman (19.3%) [18] and Malaysia (14%) [12]. Sherma et al [19] reported that non-refractive abnormalities were comparably higher than refractive conditions, which are inconsistent with our findings. Many studies reported hypermetropia to be the commonest Refractive errors in school of the deaf [20,21]. However in our study we found that astigmatism was the leading refractive anomaly (52.6%), followed by hypermetropia which is similar to studies conducted in Turkey and UK [7,22].

In our study, we did not detect any blind student, although Refractive errors were high and those who had severe visual impairment were due to high myopia mainly. Strabismus had been cited with rate of (1.3%) and (3.7%) in different studies [8,23], in the present study it was found to be (2.2%) which is quite important. Thus high prevalence of refractive and strabismic errors in those students who may be amenable to spectacle, surgical or orthoptic treatment makes early prompt diagnosis essential for this population as they are dependent upon vision for their maximal cognitive, psychological, and emotional development.

It is well known that the retina and cochlea develop from the same embryonic layer during the early embryogenic period, so oculo-auditory syndromes have been well defined in previous reports [8,22]. In the present study (10.9%) of the students were having pigmentary retinopathy (presumed to be Usher's syndrome as no ERG was done), similarly reported by Met et al in the USA where (10%) of deaf students had Usher's syndrome [24] and near to that found in Nigeria (7%),[25] but comparably higher in Malaysia (35.2%) [12]. Regarding Waardenburg syndrome (deafness with heterochromiciridum, abnormal pigmentation of skin and hair) accounted for 2% to 5% of cases of congenital deafness worldwide [22]. In this study (5.8%) of students were diagnosed with this syndrome, which is relatively

higher than that in USA (0.9%) and Southern Africa (3%) [19,26]. This high prevalence of retinal abnormalities necessitates the importance of ophthalmic and genetic consultations in these students for early diagnosis of syndromes which may be associated with other systemic abnormalities and for appropriate educational and psychological rehabilitation.

Allergic conjunctivitis was the fourth ophthalmic problem detected in (5.1%) of the students which is comparable to a result found in Nigeria (3.8%) [25]. Appropriate treatment was prescribed with explanation of the nature of the disease.

In conclusion, ocular abnormalities in deaf students are remarkably high in this study comprising (63%), which indicates the importance of prompt complete ophthalmologic examination; thus serving two principal goals. The first goal is to determine the visual acuity and identify visual deficits requiring intervention and the second is to aid in the identification of hereditary hearing loss syndromes that are associated with ocular findings. This early identification gives patients and their families the comfort of diagnosis and may provide relevant prognostic information in some diseases.

Institutions for deaf students should be aware of the high prevalence of ophthalmic disorders and the importance of vision to the development of a deaf child. Refractory errors was the commonest ocular problem 41.3%, which make complete and periodic ophthalmologic examination mandatory for every student at the time of admission and thereafter in order to correct any ametropia as soon as possible to maintain the best visual efficiency, to alleviate physical and mental isolation, improve employment opportunities as well as facilitate adequate integration of the deaf student as a useful and productive member of the society.

Ophthalmologists play an important role in organizing screening programs to facilitate the early diagnosis and treatment of related diseases. It is worth mentioning that this study may be the starting point in Yemen for the establishment of the hearing impairer's educational, social and psychological well-being in the future.

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Author Contribution

All the authors contributed in the conception and design, acquisition, analysis, and interpretation of data.

Conflict of Interest

The authors declared no conflict of interest

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Clinical and Paraclinical Predictors of Mechanical Ventilation in Guillain Barré Syndrome

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Abstract

Background Guillain Barré syndrome (GBS) is an acute post infective autoimmune polyradiculo-neuropathy; it is the commonest polyneuropathy causing respiratory failure. A lot of studies suggested certain GBS clinical and preclinical features anticipate and predicate the neuromuscular respiratory failure and can accurately assess the progression to mechanical ventilation; bulbar muscles involvement, severity of weakness of upper and lower limbs, bilateral facial muscles involvement and autonomic nervous system involvement were the main features associated with progression to mechanical ventilation.

Objectives To assess demographic, clinical and para clinical features and their relation with the progression of GBS to respiratory failure.

Methods Clinical and paraclinical predictors of impending respiratory involvement and requirement for mechanical ventilation were studied in 40 GBS patients aged 12-57 years (28 males and 12 females).

Results Ten (6 female/4 male) patients (25%) were admitted to the intensive care unit and received mechanical ventilation. Younger age, female gender and rapid disease progression in first 3 days were associated with respiratory involvement and subsequent ventilation. Bulbar weakness, bilateral facial palsy, poor digit counting (<10/1 breath) were the strongest indicators of impending respiratory failure. In combination they were found in 90% of ventilated patients. Dense weakness (power grade ≤2), weak neck flexion and axonal electromyography also showed significant risk for mechanical ventilation. Other parameters (autonomic dysfunction, antecedent gastrointestinal and respiratory illness, earlier upper limbs weakness and pain) showed no statistical significance in our study

Conclusion Respiratory failure in the course of GBS can to some extent, predicted depending on clinical information. Respiratory failure was associated with younger age, female gender, rapid progressive weakness, bulbar weakness. Facial weakness. Dense weakness, weak neck flexion, poor digit count and axonal neuropathy.

Keywords Guillain Barre syndrome, respiratory failure, bulbar weakness, mechanical ventilation.

List of abbreviation: GBS = Guillain-Barré syndrome, ICU = intensive care unit, GI = gastrointestinal, EMG = electromyography

Introduction

Guillain-Barré syndrome (GBS) is a group of autoimmune syndromes consisting of segmental demyelination and acute axonal degenerating forms [(1)]. All GBS variants are rapidly evolving polyradiculoneuropathy preceded by a triggering event, most often an infection [(2)]. GBS generally manifests as progressive areflexic weakness with or without autonomic disturbances [(1)].

Its incidence rate is 4/ 100,000 per year worldwide [(3)]. The age range was from 2 months to 95 years [(4)], with most patients presenting

between 15-50 years age [4,5,6]. Pathophysiologically peripheral nerve focal demyelination leading to conduction slowing or conduction block was the most common pathophysiological feature; however there is a rare pathophysiologic type of axonal forms [6,7].

Respiratory failure was reported in 10-30% of GBS patients which may require respiratory support by Mechanical ventilation.

Respiratory failure caused by neuromuscular dysfunctions develops rapidly in very short period mandating immediate mechanical ventilation [8].

Respiratory failure in GBS is caused by weakness of the facial, oropharyngeal, and laryngeal muscles, also weakness of the muscles of inspiration (the diaphragm, intercostals, and accessory muscles) results in inadequate lung expansion and frequently decompensation occurs during night sleep when the diaphragm affects nearly all the work of breathing [9,10]. Nevertheless expiratory-muscle weakness prevents adequate cough and secretion clearance, increasing the risk of aspiration and pneumonia [10].

The purpose of this study is to identify the demographic, clinical and paraclinical features that may help in anticipating the progression of GBS to respiratory failure

Methods

Across sectional study of forty patients with acute paralysis attended the neurology ward or intensive care unit (ICU) in Neuroscience Teaching Hospital from January to May-2012, were collected. Forty patients were examined by neurologists and considered as GBS cases when fulfilled Asbury criteria [11-12]. They were 28 males and 12 females with an age range from 12-57 years (mean = 34 years). Ten out of the total number (6 female/4 male) patients were admitted to ICU and mechanically ventilated for respiratory failure.

Time to peak disability was defined as time to intubation (patients who were ventilated), or time to the worst motor function (patients who were not ventilated) from onset of neuropathic symptoms [13].

The patients were divided into 3 groups:

1. Those who progressed to peak within 3 days (≤ 3 days).
2. Those who progressed to peak within 4-7 days.
3. Those who progressed to peak more than 7 days.

Regarding Antecedent infections, patients were divided into those with preceding GI illness (diarrhea, abdominal pain), respiratory illness (flu, cough) or those who had negative history of preceding infection.

The patients were studied in two steps; relation of all types of antecedent infection to ventilation and whether gastro-intestinal (GI) illness or respiratory illness is related to ventilation.

Each patient was examined for:

1. Bilateral facial weakness.
2. Bulbar weakness.
3. Weakness grade: weakness at presentation was graded according to medical research council scale for muscle power dense weakness was defined as grade 2 or less [14].
4. Distribution of limb weakness at presentation: upper limb weakness at presentation was taken as a parameter.
5. Autonomic dysfunction: was assessed according to Ewing method [15].
6. Weakness of neck flexion (patient fails to elevate the head against gravity or against resistance).
7. Digit count in one deep breath was taken as a rough estimate of vital capacity.

Digit count less than 10 (corresponds to vital capacity less than 1 L) was taken as a variable in prediction of mechanical ventilation [16]. The patients then were divided into 3 groups; those who count to less than 10, those who count between 10-15 and those who count to more than 15.

8. Presence of pain: whether dysasthesia or cramp muscle pain.

Electromyography (EMG) was done in whole non-ventilated patients, but only in 4 ventilated

patient. It could not be conducted in 6 ventilated patients as they were directly transferred to the ICU. It was performed after the first week of the disease. EMG study was done in the same neurophysiology clinic.

Cerebrospinal fluid examination was refused by most patients, including those admitted to the ICU. So it was canceled as a parameter. It was done in 5 patients only and was typical of albuminocytologic dissociation.

Statistical analysis

Characteristics between patients with GBS who received mechanical ventilation and those who did not were assessed using unpaired t-test with Welch correction for comparability. Fisher exact test and odd ratio for categorical variables by contingency table using Graph Pad in Stat 3 Software, Version 3.06 [17] was used to assess the p value. P value < 0.05 was regarded statistically significant to the prediction of ventilation.

Results

Twenty seven patients (68%) gave history of a preceding infection. Nine of them underwent ventilation. No statistical significance for the presence of a preceding infection to mechanical ventilation No statistical significant difference between antecedents GI or respiratory illness to ventilation.

Bilateral facial palsy (whether symmetrical or asymmetrical) was seen in 14 patients (35%), 9 of them (64.3%) needed ventilation ($P < 0.0001$) which indicates a considerable significance association of bilateral facial weakness with ventilation (Table 1).

Bulbar weakness was seen in 15 patients (38%). Mechanical ventilation was indicated in 10 of them (all ventilated patients). Therefore, bulbar weakness was a considerably significant predictor for mechanical ventilation ($P <0.0001$) (Table 1).

Dense weakness (power grade ≤ 2) at presentation was studied for the prediction of mechanical ventilation.

Eighteen patients (45%) had dense weakness at presentation. Eight of them (44%) were ventilated ($P = 0.02$, which is a statistically significant suggesting association of severe weakness with mechanical ventilation (Table 1).

Distribution of weakness at presentation was studied to predict the progression to mechanical ventilation.

Upper limbs weakness presented in 13 patients (32.5%); 5 of them needed ventilation ($P = 0.2$). Three patients gave history of simultaneous upper and lower limbs weakness; 2 of them were ventilated.

Twenty-five patients (63%) were having autonomic dysfunction, 9 of them (36%) needed ventilation ($P = 0.06$). Twenty patients were having weak neck flexion; half of them were ventilated ($P = 0.0004$) (Table 1).

Patients were divided into 3 groups according to their ability to count in one deep breath. Ten patients (25%) could count to (<10) in one breath; Nine of them (90%) were ventilated ($P < 0.0001$). Pain was reported in 22 patients (55%). Only 4 of them (18.2%) needed ventilation ($P = 1.0$).

Six patients out of 34 patients in whom EMG was done; were having axonal pattern (18%), 3 of them (50%) needed ventilation ($P = 0.01$) showed that axonal pattern is a significant risk to mechanical ventilation (Table 1).

In this study, only 2 patients were having a previous attack of GBS. Both were female, having mild disease (able to walk), none of them needed ventilation.

Two females in the study were pregnant. One 14 years old, was in the last trimester and she did not need ventilation. The second pregnant female was 24 years old, was in the first trimester and needed ventilation within 2 days of the onset.

Table 1. Baseline Demographics and Clinical Features of Guillain-Barré syndrome Patients

Variables		Mechanical ventilation		*P* value
		Yes (N = 10)	Not (N = 30)	
Age (yr) mean (range)		18 (14-24)	27 (12-57)	0.0005
Gender (female/Male)		6/4	6/24	0.04
Time to peak disability (≤3)		7 (70%)	5 (17%)	0.003
Facial weakness		9 (90%)	5 (17%)	< 0.0001
Bulbar weakness		10 (100%)	5 (17%)	< 0.0001
Dense limbs weakness		8 (80%)	10 (33%)	0.02
Upper limb weakness		5 (50%)	8 (27%)	0.2
Autonomic dysfunction		9 (90%)	16 (53%)	0.06
Weak neck flexion		10 (100%)	10 (33%)	0.0004
Digit count < 10		9 (90%)	1 (3%)	< 0.0001
Pain		6 (60%)	16 (53%)	1.0
EMG (axonal)		3 (30%)	3 (10%)	0.01
Antecedent infection	GI illness	5 (50%)	9 (30%)	0.1
	URTI	4 (40%)	9 (30%)	
	Total	9 (90%)	18 (60%)	

GI = gastrointestinal, URTI = upper respiratory tract infection, Two tailed P value by unpaired t- test and Fisher exact test

A quarter of the patients received mechanical ventilation and (75%) did not. The youngest patient in the study was 12 years old and the oldest was 57 years. For ventilated patients, mean age was 18; and for non-ventilated it was 27 (Table 2).

Table 2. Relation of age to ventilation

Mechanical Ventilation	No.	Age (yr) mean±SD
Yes	10	18.00±3.71
No	30	27.06±11.39

P = 0.0005, 95% confidence interval (95%CI) = -16.543 to -1.590

Females were 30% (12/40) of the total number, and 60% (6/10) of ventilated patients were females. There was a significant association between female gender and respiratory failure (*P* = 0.04) (Table 3).

Progression to peak disability ranged between 1-15 days (mean was 5.4 days). Mean progression to peak disability for ventilated patients was 4.6 days, and for non-ventilated 5.7 days. Patients were divided into 3 groups regarding progression to peak disability (Table 4). Seventy percent of ventilated patients progressed to peak within 3 days. This study showed a statistical significance to rapid progression (≤3 days) for ventilation (*P* = 0.003) (Table 4).

Table 3.Gender and ventilation in Guillain-Barré syndrome patients

Gender	Mechanical ventilation		Total
	Yes	No	
Male	4 (10%)	24 (60%)	28 (70%)
Female	6 (15%)	6 (15%)	12 (30%)
Total	10 (25%)	30 (75%)	40 (100%)

P = 0.04, Odd ratio = 0.16, 95%CI = 0.03538 to 0.7851

Discussion

The results of this study suggest that the failure of neuromuscular respiratory function and progression to mechanical ventilation should be anticipated in GBS patients by assessing certain clinical and preclinical features.

Peak of age in whole patients was in the second decade (45%). Seven ventilated patients (70%) were in the second decade and 3 patients (30%)

in the third decade. Younger age is strongly related to the risk of ventilation in the present study.

Table 4. Three groups of patients regarding progression to peak disability

Progression (days)	Mechanical ventilation		Total
	Yes	No	
≤ 3	7	5	12
4 -7	1	18	19
> 7	2	7	9
Total	10	30	40

P = 0.003, Odd ratio = 11.66, 95% CI =2.220 to 61.303

Although males were more than females in whole patients by 2.6:1 ratio (consistent with other studies) [11], ventilated females were more than ventilated males by 3:2 ratio and statistical study showed a significant correlation between female gender and ventilation.

The above 2 findings (age and gender) were not fit with Lawn et al study, which showed no significant difference between those patients who received ventilation and those who did not for age and gender [11]. Lawn et al studied 114 patients of different ages and relatively equal number of patients to both genders, admitted to ICU over 20 years, whereas the present study assessed the patients over 4 months with age between second and third decade and relative male predominance.

Progress to peak disability within 3 days was a significant prognostic factor for respiratory failure and subsequent ventilation. Seven out of the ten patients who were ventilated (70%) were progressed to peak disability within 3days. The association between rapid progression and the likelihood of mechanical ventilation was also noticed in Lawn, et al study [11]. This feature might be a predictor of the fulminant course of the disease.

According to the present study, history of antecedent infection showed no association with the mechanical ventilation, and there is also no significance association with antecedent GI illness or to respiratory illness as a risk factor for ventilation. This is in agreement with Lawn et al study [11] and Al-Zaidi study [18]. Some previous reports considered antecedent GI illness as a bad prognostic point [11].

Autonomic dysfunction was identified in a high proportion of patients who subsequently received mechanical ventilation (90%) but this did not reach statistical significance. This finding is in agreement with Lawn et al study [11], but against Al-Tamimi study [12] who found a significant correlation between autonomic dysfunction and subsequent mechanical ventilation [20]. The present study and Lawn, et al study depend on Ewing criteria (appendix III) to define autonomic dysfunction; while Al-Tamimi study [12] (on autonomic dysfunction in GBS) depend on development of any clinical sign of autonomic dysfunction.

Weakness of neck flexion was reported by many studies [11], our study also found a strong indicator for the likelihood of subsequent ventilation. This could be related to the fact that neck flexion has the same root innervations as the diaphragm [6].

The present study showed no relation between pain and the subsequent need of ventilation. Axonal pattern EMG was seen in 3 of 4 ventilated patients and 3 of 30 non-ventilated patients. It was a highly significant predictor of mechanical ventilation. Lawn et al found that axonal EMG was associated with an adverse outcome, but not specifically in predicting ventilation [11].

One of two pregnant ladies with GBS in our study developed respiratory failure and received mechanical ventilation. They had progressed to respiratory impairment within 2 days with early development of bulbar and facial weakness. This may point to fulminant course of GBS in pregnant women [19,20]. This requires a separate study to estimate the risk of ventilation in pregnant women with GBS.

Two patients in our study had a previous attack of GBS years ago. They comprise (5%) of whole

patients in the study, which may approximate the percent of GBS recurrence in most reports [3]. In conclusion, while inherently unpredictable, the course of patients with GBS can, to some extent, be predicted on the basis of clinical information and simple bedside tests of respiratory function. These data may be used in the decision regarding admission to the intensive care unit, preparation for elective intubation, and possible mechanical ventilation.

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Author Contribution
Author ZAKI NOAH HASAN designed the study, performed in statistical analysis, wrote the protocol and wrote the first draft of manuscript, managed the analysis of the study and managed the literature search. Author Sajid Ibrahim wrote and revised the first and the final draft of manuscript . The third and fourth authors collect the patients for the study and read and approved the final manuscript.

Conflict of Interest
Authors have declared no conflict of interests.

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Erectile Dysfunction in Haemodialysis Patients in Al-Imamain Al-Kadhemain Medical City and Al-Kindy Teaching Hospitals

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Abstract

Background Erectile dysfunction is the inability to attain or maintain an erection sufficient for satisfactory sexual performance. There is a very high prevalence among dialysis patients. Many factors play a role including the disease itself and dialysis.

Objective To identify the rate of erectile dysfunction in uremic patient undergoing haemodialysis and to find the association between the erectile dysfunction and many confounding factors.

Methods All male patients of end stage renal disease were kept on maintenance haemodialysis therapy. Patients were divided into two groups according to the International index of erectile function-5, first group with erectile dysfunction with score of 21 and less, the second group without erectile dysfunction with score of 22 and more.

Results The percentage of erectile dysfunction in the study sample was (84.9%). Factors responsible for erectile dysfunction are diabetes mellitus (73.3%), increasing age (75.5%) of patients, high predialysis urea level (82.2%), smoking, hepatitis B and C virus infection, systolic blood pressure, albumin, creatinine, haemoglobin and the duration of dialysis are not related.

Conclusion Majority of the patients with end stage renal disease on maintenance haemodialysis have higher rate of erectile dysfunction. Major factors responsible for erectile dysfunction are diabetes mellitus, increasing age and high predialysis urea.

Keywords Haemodialysis, erectile dysfunction, International index of erectile function-5.

List of abbreviations: CKD = Chronic kidney disease, ED = Erectile dysfunction, DM = diabetes mellitus, Hbsag = Hepatits b surface antigen, HCV ab = Hepatitis C virus antibody, HD = Hemodialysis, IIED-5 = International index of erectile function.

Introduction

Chronic kidney disease (CKD) in adults is defined by The Kidney Disease Outcomes Quality Initiative (K/DOQI) as structural and/or functional kidney abnormalities (abnormal urinalysis, imaging studies, or histology) that persist for at least three months [1-2]. CKD has been classified into five stages according to the degree of the glomerular filtration rate [3-4]. Sexual dysfunction in CKD includes erectile dysfunction (ED), decreased libido and marked decrease in the frequency of intercourse [5].

ED is defined as the consistent or recurrent inability to acquire or sustain an erection of sufficient rigidity and duration for sexual intercourse [6]. In general, ED is present in up to 30 million men in the United States and approximately 100 million men worldwide [70]. Patients of CKD have prevalence of ED ranging from approximately 50 to over 90% [8,9]. ED may result from three basic mechanisms failure to initiate, to fill and to restore the function of cavernous muscle [10].

In addition to age, the best predictors of ED are diabetes mellitus (DM), hypertension, obesity, dyslipidemia, cardiovascular disease, smoking, and use of medication, psychosocial disease, neurological and endocrine disorder [11-13]. The association of cardiovascular disease and ED is due to sharing many risk factors and both pathophysiology is mediated through endothelial dysfunction [14-16]. The management of uremic men with sexual dysfunction begins by maximizing the delivered dose of dialysis, discontinuing medications (if possible), correcting the anaemia of chronic renal disease, as an example, the administration of recombinant human erythropoietin to raise the hematocrit to 33 to 36 percent may enhance sexual function [17].

Different treatment strategies known at the time being for treatment of ED; phosphodiesterase inhibitors l, Psychotherapy and/or psychoactive medications [18-20].

The aim of the study to identify the prevalence of ED in uremic patients undergoing haemodialysis and to find the relationship between the ED and certain confounding factors.

Methods

A descriptive case – series study conducted from the 1st of November 2011 to the 31st of January 2012 in the Haemodialysis unit of Al-Imamain Al-Kadhemain Medical City and Al-Kindy Teaching Hospital in Baghdad city.

Fifty-three male patients who were on regular maintenance haemodialysis were included in the study. Only those patients who had live spouses were included. Their age range was 18 to 75 year. The marital sex is considered as an appropriate expression of sexuality.

Exclusion criteria: Patients of acute renal failure and those with cognitive/communication deficit.

All patients were informed and consent about the study was taken. Each subject completed a self-administered 5-item validated questionnaire [21], the International Index of Erectile Function (IIEF-5), adapted in Urdu [22] which is a bridged version of the 15-item International Index of Erectile Function [23]. On the basis of IIEF-5, categorisation of ED was done into those with the ED (with score of 21 and less) and without ED for those with score 22 and more (total score =25).

Data was analysed dividing the patients into ED and None ED groups. Demographic data was collected on a forma containing age, duration of dialysis, history of smoking and of DM. At the same time blood pressure was checked and blood sample of these patients was drawn to measure blood (urea, creatinine, blood sugar, albumin, HBs Ag, Anti HCV).

Data was entered and analysed using SPSS 16.0. Mean±SD is given for normally distributed quantitative variables. Frequencies and percentages are given for qualitative variables. Pearson Chi square test was applied to observe correlations in qualitative variables. A P <0.05 was considered statistically significant.

Results

Table 1 show the demographic data of the 53 patients studied that includes the mean of the age of patients was 38.79 ± 9.03 years. IIEF-5 score: 45 patients (84.9%) have IIEF-5score less than 22 (they have ED), 8 patients (15.1%) have IIEF-5 score equal to or more than 22 (they did not have ED).

The mean age of those patients with ED was 38.93 ± 8.84 years, which is significantly higher (P < 0.05) than 30.63 ± 4.17 years of patients without ED. The mean urea level for patients with ED was 179.09 ± 32.03 mg/dl, which is significantly higher (P < 0.05) as compared 138.9 ± 38 mg/dl for patients without ED.

Concerning albumin, systolic blood pressure, creatine level, Hb level and mean duration of dialysis, they were not different between the two groups (Table 2).

Table 1. Demographic data of the patients included in the study

Feature	Category	No.	%	Mean
Age (years)	< 35	17	32.1%	38.79 ± 9.03
	35-44	24	45.3%	
	≥ 45	12	22.6%	
Urea level (mg/dl)	< 150	14	26.4%	173.0 ± 35.69
	150-189	21	39.6%	
	≥ 190	18	34.0%	
Albumin level (g/dl)	< 2.5	14	26.4%	2.95 ± 0.64
	2.5-3.4	26	49.1%	
	≥ 3.5	13	24.5%	
Systolic BP (mmHg)	< 140	4	7.5%	168.8 ± 25.1
	140-179	23	43.4%	
	≥ 180	26	49.1%	
Creatinine (mg/dl)	< 4	2	3.8%	6.65 ± 1.53
	4-7.9	38	71.7%	
	≥ 8	13	24.5%	
Duration of dialysis (months)	< 17	24	45.3%	19.91 ± 7.32
	17-22	17	32.1%	
	≥ 23	12	22.6%	
Hb (g/dl)	< 7	4	7.5%	9.28 ± 1.77
	7-10	39	73.6%	
	≥ 10	10	18.9%	
Diabetes mellitus	Yes	34	64.2%	---
	No	19	35.8%	
HBsAg state	Negative	47	88.7%	---
	Positive	6	11.3%	
HCVab state	Negative	38	71.7%	---
	Positive	15	28.3%	
Smoking	Non smoker	23	43.4%	---
	ex-smoker	22	41.5%	
	smoker	8	15.1%	
IIEF-5 score	< 22	45	84.9%	---
	≥ 22	8	15.1%	

BP = Blood pressure, IIEF-5 = International Index of Erectile Function

Table 2 showed quantitative variables of patients found with and without ED. Thirty three patients who were diabetics at the same time presented with ED. A value that significantly higher (P <0.05) than only uremic patients who had no ED. Concerning smoking habit, HBsAg positive and anti HCV positive, no significant difference was noted between those with and without ED (Table 3).

Table 4 show relationship between ED and certain confounding factors It was evident that blood urea, Diabetes mellitus and the age of patients were significantly associated with ED (P < 0.05), on the contrary albumin level, creatinin level, systolic blood pressure, Hb level, smoking

habit, HBsag state and HCVab state showed no significant association with ED.

Table 2. Quantitative variables of patients with and without erectile dysfunction

Feature	Erectile dysfunction		*P* value
	Yes (Mean ± SD) N = 45	No (Mean ± SD) N = 8	
Age (years)	38.93 ± 8.48	30.63 ± 4.17	< 0.05
Urea level (mg/dl)	179.09 ± 32.03	138.9 ± 38	< 0.05
Albumin level (g/dl)	2.91 ± 0.62	3.61 ± 0.51	> 0.05
Systolic BP (mmHg)	170.2 ± 25.5	161.2 ± 22.9	> 0.05
Creatinine (mg/dl)	6.47 ± 1.47	7.71 ± 1.53	> 0.05
Duration of dialysis (months)	19.73 ± 7.55	20.88 ± 6.20	> 0.05
Hb (g/dl)	9.16 ± 1.79	9.92 ± 1.65	> 0.05

BP = Blood Pressure, Hb = Hemoglobin

Table 3. Qualitative variables of patients with and without erectile dysfunction

Variable		Erectile dysfunction		*P* value
		Yes (Mean ± SD) N = 45	No (Mean ± SD) N = 8	
Current smokers		7	1	< 0.05
HBsag positive		5	1	> 0.05
Anti HCV positive		13	2	> 0.05
Diabetes mellitus	Yes	33	1	< 0.05
	No	12	7	

DM = diabetes mellitus, ED = erectile dysfunction

Discussion

ED is a major health issue in modern life, impact on quality of life [24]. In this study, there is very high prevalence (84.9%) of ED in haemodialysis patients, which is similar to that observed in Iran (87.5%) [25], Turkey (82.9%) [26], Egypt (82.5%) [27] and Brazil (86.4 %) [28]. Factors responsible for such a high rate of ED in dialysis patients in current study is related with multiple factor including DM, increasing age (more than thirty five years) and very high pre dialysis urea level.

The current study showed that the ED was more prevalent in diabetic than non diabetic patients and it reveals significant association. Similar result is observed by Miyata et al [29]. DM affects ED in many ways. Large vessel atheromatous disease is 40 times more prevalent amongst men with diabetes compared to non-diabetics [30]. DM causes ultra structural changes in cavernosal tissues, these changes including reduction in smooth muscle content, increased collagen deposition, thickening of basal lamina and loss of endothelial cells [31], and finally endothelial and neurogenic relaxant responses mediated by nitric oxide are impaired in diabetes mellitus [31,32].

In the current study, increasing age significantly correlated with the prevalence of ED, which is in agreement with other studies like the Massachusetts Male Aging (MMA) study [33], Rodger et al [34], Chun–Fu Lia et al [35] and Rosas et al [36] found a strong association between age and prevalence of ED.

Table 4. The relationship between the urea, albumin, SBP, creatinine, duration of the dialysis, age, Hb and erectile dysfunction

Parameters		With ED		Without ED		Total		*P* value
		No.	%	No.	%	No.	%	
Age (years)	< 35	11	24.4	6	75	17	32.1	< 0.05
	35-44	23	51.1	1	12.5	24	45.3	
	≥ 45	11	24.4	1	12.5	12	22.6	
	total	45	100	8	100	53	100	
Duration of haemodialysis (months)	< 17	21	46.7	3	37.5	24	45.3	> 0.05
	17-22	14	31.3	3	37.5	17	32.1	
	≥ 23	10	22.2	2	25	12	22.6	
	total	45	100	8	100	53	100	
Urea level (mg/dl)	> 150	8	17.8	6	75	14	26.4	<0.05
	150-189	20	44.4	1	12.5	21	39.6	
	≥ 190	17	37.8	1	12.5	18	34.0	
	total	45	100	8	100	53	100	
Albumin level (g/dl)	<2.5	12	26.7	2	25	14	26.4	<0.05
	2.5-3.4	23	51.1	3	37.5	26	49.1	
	>=3.5	10	22.2	3	37.5	13	24.5	
	total	45	100	8	100	53	100	
Systolic BP (mmHg)	< 140	3	6.7	1	12.5	4	7.5	<0.05
	140-179	19	42.2	4	50	23	43.4	
	≥ 180	23	51.1	3	37.5	26	49.1	
	total	45	100	8	100	53	100	
Creatinine level (mg/dl)	< 4	2	4.4	0	0	2	3.8	<0.05
	4-7.9	34	75.6	4	50	38	71.7	
	≥ 8	9	20	4	50	13	24.5	
	total	45	100	8	100	53	100	
Hb level (g/dl)	< 7	4	8.9	0	0	4	7.5	>0.05
	7-10	33	73.3	6	75	39	73.6	
	≥ 10	8	17.8	2	25	10	18.9	
	total	45	100	8	100	53	100	

The average age of this patient with ED was 50 years and 38 years for those without ED (P < 0.001). While in the present study, the mean of the age of patients with ED was 38.9 years and 30.6 years for patients without ED. This difference may be related to other factors like dialysis techniques, medications (like antihypertensive medications) and psychological state.

Age causes gradual changes in sexual organs; these changes do not occur suddenly like women but occurs gradually during a process called andropause or late onset hypogonadism [37]. An abrupt increase in hypogonadism prevalence occurred in men aged 45 to 50 years beyond which a plateau of prevalence was maintained until older than 80 year of age [38]. In present study, the blood urea level in patient with ED was higher in those with ED; similar result was observed by Mumtaz et al [39]. Increased urea level leads to decreased synthesis of nitric oxide and super saturation of the oxygen free radicals, these oxygen free radicals lead to increased consumption of nitric

oxide, which is a relaxing factor for penile smooth muscles [40].

Table 5. The relationship between the HBs Ag, HCVAb, diabetes mellitus, smoking and erectile dysfunction

Parameters		With ED		Without ED		Total		*P* value
		No.	%	No.	%	No.	%	
HBsag state	Negative	40	88.9	7	87.5	47	88.7	> 0.05
	Positive	5	11.1	1	12.5	6	11.3	
	Total	45	100	8	100	53	100	
HCVab state	Negative	32	71.1	6	75	38	71.7	> 0.05
	Positive	13	28.9	2	25	15	28.3	
	Total	45	100	8	100	53	100	
Diabetes mellitus	No	12	26.7	7	87.5	19	35.8	< 0.05
	Yes	33	73.3	1	12.5	34	64.2	
	Total	45	100	8	100	53	100	
Smoking	Non smoker	19	42.2	4	50	23	43.4	> 0.05
	Ex-smoker	19	42.2	3	37.5	22	41.5	
	Smoker	7	15.6	1	12.5	8	15.1	
	Total	45	100	8	100	53	100	

In the present study, the systolic blood pressure and serum albumin level show no significant association with ED ($P > 0.05$), this result is comparable to Mumtaz et al study [39].

The mean duration of dialysis, s. creatinin level and Hb level, smoking habit and HBsag have nothing to do with ED, findings in accordance with Steel et al [40-41], Messina et al [42], Leila et al [43], and Mumtaz et al [39] current study the HBsag state has non significant association with ED.

In conclusion, majority of the patients suffering from ED, on maintenance haemodialysis are having ED, haemodialysis does not improve sexual dysfunction, and major factors responsible for ED are diabetes mellitus, age more than 35 years and high pre dialysis urea.

The limitation of the study was lack of control group; follow up was not done which would have been useful to determine small size, and assessment of sex hormones

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Author contribution

The first author involved in the collection of samples, arrangement and writing of the study under the supervision of the second author.

Conflict of interest

The authors declare no conflict of interest.

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Pedicle Screw Placement versus Classic Surgery in Lumbothoracic Spine Disorder

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Abstract

Background Pedicular screw fixation surgery for thoracolumber disorders is well established surgical method to treat instability due to different etiologies due to trauma, infection, tumor as well as spondylopathic deformity.

Objective To evaluate surgical modalities in the treatment of lumbothoracic disorders.

Methods Prospective study of 30 patients 6 lower dorsal trauma, 2 treated by screw and 4 by decompressive laminectomy and bone graft using rib, 4 spondylolesthsis treated by screw and 8 cases by decompessive laminectomy and fusion, 4 spondylosis treated using screw and 8 treated only decompressive and foraminotomy .

Result Spinal fusion using pedicle screws has become popular worldwide in treating a variety of disorders of the spine. Treatment of thoracolumbar fracture with pedicle screws at injury level is easy and worthy. Compared to the lumbar region, the insertion of thoracic pedicle screws remains a challenge, despite of modern technology and computer assistance especially in the upper thoracic spine, where misplacement rates of up to 40% CT-navigation leading to the conclusion that pedicle screw instrumentation in the middle and upper thoracic area should be carried out with the help of navigation only. The availability of an intraoperative CT seems to be of particular importance. An accurate assessment of screw positions becomes hereby possible without any significant time delay and with utmost accuracy.

Conclusions Transpedicular fixation of thoracolumbar and lumbar spine fractures has become a frequently used technique. Transpedicular screw fixation provides the greatest stability in the unstable spine.

Keyword Pedicle screw, accuracy, lower dorsal trauma, hydatid spine

List of Abbreviation: CT = computerized tomography, AP = antero-posterior, PSD = pedicular screw diameter, TSA = transverse section angle, MRI = magnetic resonance image.

Introduction

Pedicle screw fixation use for spinal instability was first reported for the lumbosacral region and has been extensively studied and is widely performed today [1].

The relative ease of implantation is mainly due to the larger size of both the vertebral body and the pedicle diameters, as compared to the mid and upper thoracic vertebral anatomy [2] Interest in thoracic pedicle screw use has gained momentum recently, especially in the lower thoracic spine [3,4].

Lumbar spinal fusion is a commonly performed surgical procedure. It is used in a variety of spinal pathologies including degenerative disease, trauma, spondylolisthesis and deformities.

A mechanically stable spine provides an ideal environment for the formation of a fusion mass. Though the degree of stability required for spinal

fusion is unknown, increased stiffness of the spine improves fusion rates, and lowers the chances of nonunion at the graft site. Instrumented spinal fusion also allows early ambulation with minimal requirement of a post operative external immobilizer [5,6]. The first attempt at spinal fusion with internal fixation was reported in 1891 [5] with the use of a wiring technique. Currently, pedicle screws are frequently used to provide spinal stability till the formation of a fusion mass. Pedicle screw fixation has numerous advantages over other methods of spinal fixation for the last two decades points towards their efficacy and consistency in outcomes ([7-9].

Concerns have been raised regarding the extensive paraspinal muscle retraction required for their insertion, and the consequent increased infection rates and muscle injury [10]. Also improperly placed screws may cause neural and vascular damage [11].

Small pedicle width, altered pedicle morphology, and shift of the surrounding structures by rotation causes a consistently smaller safe zone in terms of pleural, spinal cord, and vascular injury. In thoracic pedicle fixation of pediatric idiopathic scoliosis [12,13] however, despite their common use, safety concerns related to screw malposition have been described [14-16].

Violation of the pedicle by a screw can cause injury to the neural structures along any of the four quadrants of the pedicle. When this occurs, the negative consequences of screw placement may outweigh the advantages offered by the systems [17].

The objective of this study was to evaluate different surgical modalities used in the treatment of lumbothoracic disorders and study the percent of accuracy of pedicular screws placement

Methods

This is a Prospective study of 30 patients in Al-Kadhimmiya neurosurgical centre from Feb. 2011 to Jan. 2013. In this study, the patients diagnosed and then treated by surgery we used either decompression with laminectomy, with or without pedicle screw in lumbar spine or thoracotomy and rib graft or pedicle screw in trauma. In pedicle screw, we use these steps, pedicle preparation, determination of screw length, screw placement, rod placement, lever placement. Using the distractor for reduction and then tightening of pedicle. In cases of screw, we use special operative table and C- Arm X-Ray that enable us to take AP and lateral view of the pedicle. Follow up of patients clinically, X-Ray of spine and or CT spine in cases of pedicle screw to determined accuracy of screw.

Criteria of pedicle screw placement were:

(1) Relation of pedicle screws to the pedicle.

(2) Relation of pedicle screws to the vertebral body.

Pedicle screws are scored as follows:

Grade Ia: optimally placed screws, rigidly anchored within the pedicle and vertebral body.

Grade Ib: screws are placed with > 50% of the pedicle screw diameter (PSD) lateral outside of the pedicle and with > 50% of the PSD within the vertebral body.

Grade IIa: screws are placed with ≥ 50% of the PSD within the pedicle and > 50% of the PSD is lateral outside of the lateral cortex of the vertebral body.

Grade IIb: screws are placed with ≥ 50% of the PSD within the pedicle and the tip of the screw crosses the midline of the vertebral body.

Grade IIIa: screws are located with > 50% of the PSD lateral outside of the pedicle and the lateral vertebral cortex.

Grade IIIb: screws are located with > 50% of the PSD medial outside of the pedicle and the tip of the screw crosses the midline of the vertebral body.

The need for revision of pedicle screws was estimated on consensus of the participating surgeons.

In spinal hydatidosis, we use thoracotomy, then dorsal corpectomy, we resects rib and use it as graft. In this study, we use the term decompressive laminectomy when we perform laminectomy, disctomy, foraminotomy, removal of lateral recess either all these procedure or some of them.

Result

Second lumber wedge fracture was shown by plain X-ray (Fig. 1) and MRI (Fig. 2). Pedicular screws were fixed with rods or not (Fig. 3).

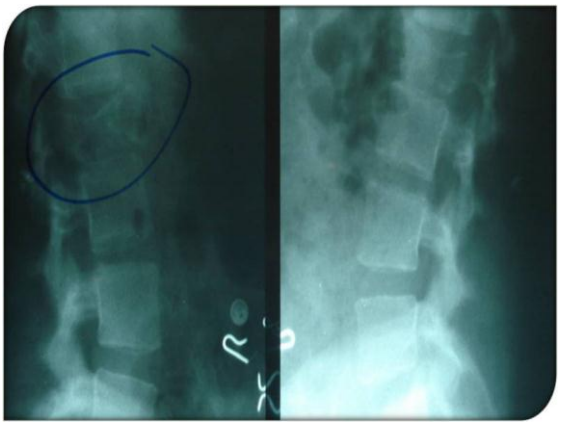

Fig. 1. Plain x-ray of lumosacral spine showing L2 wedge fracture

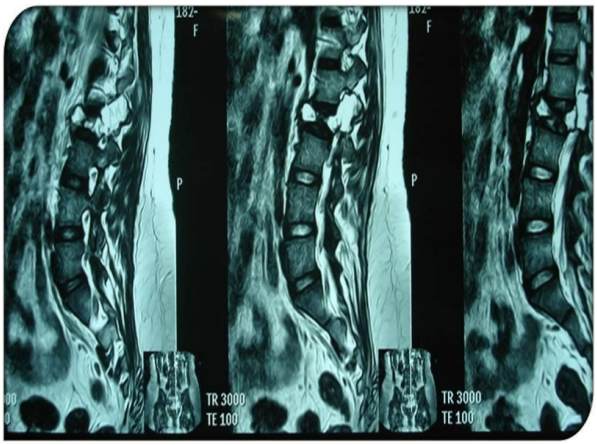

Fig. 2. Lumbosacral MRI-T2 study showing L2 fracture

Surgery done for all patients, classical laminectomy done for 8 patients and pedicular screw placement for 4 patients and in patients with spondylosis, 8 patients with classical laminectomy and 4 patients with pedicular screw, in thoracic region pathology, 2 pedicular screw surgery for traumatic injury and 4 thoracotomy and rib graft for patients with hydatid spine (Table 1).

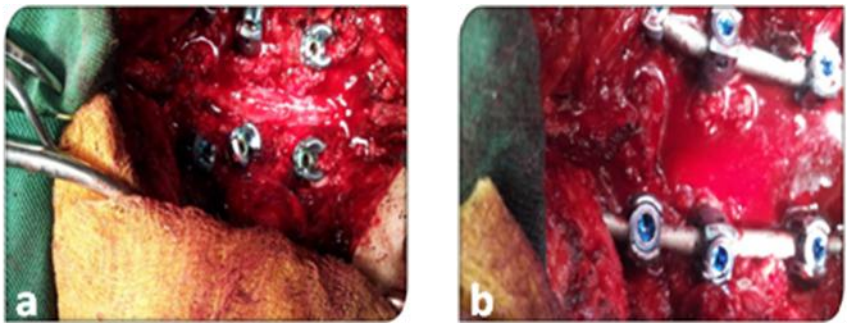

Fig. 3. Intraoperative picture showing (a) placement of pedicular screws with decompressive laminectomy, (b) placement of pedicular screws with rods for fixation.

Table 1. Distribution of patients according to the pathological disorder and type of operation

Character		Lumbar spondylosis	Lumber spondylolesthesis	Dorsal trauma	Dorsal hydatid
Number		12	12	2	4
Operation	Classical laminectomy	8	8	-	-
	Pedicle screw	4	4	2	-
	Thoracotomy and rib graft	-	-	-	4

Postoperative complications were presented in table 2. One patient with classical laminectomy had sphenicteric problem, 1 patient with lumbar screw surgery had ipsilateral lower limb parasthesia and 4 patients with thoracotomy had lower limb motor weakness preoperatively and continued postoperatively.

Table 2. Operative complications

Treatment type		Motor	Sensory	Sphinecteric
Lumber	Classic laminectomy Screws	Nil Nil	Nil Paraesthesia	1 Nil
Dorsal	Thoracotomy Screw	4 Nil	Nil Nil	1 Nil

The accuracy of pedicular screw using post operative CT scan as scoring system (42 screw of ten patients)shows that 14 patients with 1A score, 10 with 1B score, 12 with score 2A, and 6patients with score 2B but none with 3A and 3B.

Discussion

A variety of conditions resulting from degenerative, traumatic, and other abnormalities of the lumbar spine are best managed by achieving spinal stability and attaining a solid fusion. To decrease failures in arthrodesis, a number of different devices have been developed to provide internal stability while the fusion is healing.

Because the pedicle offers the strongest point of attachment to the spine, most spinal instrumentation systems use screws for fixation placed into the pedicle and then the vertebral body. However, a number of complications associated with pedicle screw fixation have been reported. One of the most serious complications related to pedicle screw usage is neurologic injury, secondary to misplaced pedicle screws abutting, or injuring, a nerve root [18].

There are a number of techniques described to determine the location to enter the posterior aspect of the pedicle. The three we chose are often discussed and used (Roy-Camille, Magerl, and Du). Roy-Camille and Magerl suggest that the starting point for inserting a pedicle screw should be based on the anatomic relation of the facet joint and transverse process [19].

Roy- Camille's entry point is the closest to the midline of the spine as the line through the plane of the facet joint is one of the crossing points [20]. Magerl's entry point is further lateral, located at the nape of the neck of the superior articular process. Du's described entrance to the pedicle is located between Roy-Camille and Magerl's [21].

Pedicle violations by pedicle screws have been reported to occur more often through the medial and lateral walls than the superior and inferior walls [22]. One reason for this is that the pedicle heights are often greater than widths. Additionally, the cortical thickness of the superior and inferior walls is generally more than 2 mm, whereas the medial and lateral walls are less than 2 mm.

If different starting holes are chosen to enter the pedicle, the angle of insertion will differ and can lead to a relative diminution in the safe range for pedicle insertion through the isthmus of the pedicle. The key to a successful transpedicular screw insertion is that the small pedicle is correctly entered, and the walls not penetrated by Du are closest to the pedicle access at L1 and L2.

There were significant differences in the safe range of TSA between the three methods from L3 to L5, as the pedicle diameters and pedicle axis increase in obliquity, while the facet joints become more coronal. The latter is used as one point in the anatomic localization of the posterior pedicle. Both Du and Magerl's techniques can be applied at L3 and L4 because of their larger safe range of TSA. At L5, however, Magerl's method is a better choice because of

the larger safe range of TSA. Roy-Camille's described technique gives the least amount of freedom in insertion angle of a pedicle screw as its safe range for TSA is the smallest.

In summary, choosing the proper entry point to inserting pedicle screws is penetration of the pedicle wall. Understanding pedicle angles and morphometry also helps decrease the risk of pedicle violation during screw insertion [23,24].

The technique utilized in this study involved a short-segment construct with pedicle screws one level above and one level below the fracture site only, while other use it with additional screws fixed at the level of the fracture.

The supporting point was set at the fractured vertebra body, which was repositioned with the appropriate connected vertebral bodies and stabilized by forces from the ligament and annulus fibrosus. In addition, the pedicle screw at the level of the injury may apply pressure to the fractured vertebral body in order to correct vertebral deformity and lateral displacement. This group found that the six screw model increased stiffness in axial loading.

In flexion testing, the six screw model demonstrated 84 % greater stiffness compared to the four screw construct. Furthermore, the six screw construct was 38 % stiffer than the four screw construct in torsional testing. Shen *et al* [25] have also suggested that six pedicle screw fixation is superior to four pedicle screw fixation. The intermediate screw is thought to function as a push point with an anterior vector, thus creating a lordotic force.

The intermediate screw also provides improved ''three point fixation,' In conclusion, under our experimental conditions, they found similar stability between the six pedicle screw model at the level of the injured vertebrae and the four pedicle screw model as we did in this study.

Additional screws placed at the fractured vertebra body may help reduce stress at both the superior and inferior pedicle screws, and may also disperse the stress load maintained by internal fixation, thus reducing screw fatigue and breakage. This study was limited to vertebral compression fracture, and thoracolumbar burst fractures.

Pedicle screw fixation is a challenging procedure in thoracic spine, as inadvertently misplaced screw has high risk of complications. The accuracy of pedicle screws is typically defined as the screws axis being fully contained within the cortices of the pedicle The use of thoracic pedicle screw instrumentation has become increasingly widespread in the treatment of scoliosis owing to the consistently superior results achieved in terms of fixation and deformity correction [26,27].

The diameters of screws were 4.5 or 5.5 mm. The accuracy of pedicle screws is typically defined as the screws axis being fully contained within the cortices of the pedicle [28,29].

The correct pedicle insertion is the aim of all surgeons so there are many scoring system ranging from free hand insertion to 3D Image, Correctly placed screw completely inside the pedicle with no breach or perforation of the pedicle wall. Minor perforation of the pedicle wall less than 2 mm to either side. Moderate displacement perforation of 2 mm to less than 4 mm to either side. Severe displacement perforation of more than 4 mm to either side of the pedicle but in this study no place for free hand pedicle screw.

The free hand pedicle screw insertion technique exhibits similar accuracy in experienced hands as compared to the image-guided techniques. It has been suggested that slightly medial 2 mm or lateral 6 mm violations have little clinical or anatomic consequence and, therefore, have been deemed as acceptably placed screws [30,31].

Medial screw malposition was measured between medial pedicle wall and medial margin of the pedicle screw. The distance between the lateral margin of the pedicle screw and lateral vertebral corpus was measured as lateral malposition.

A screw that violated medially greater than 2 mm was rated as an ''unacceptable screw'' while a screw that violated laterally greater than 6 mm was rated as an ''unacceptable screw [23]. Other they use the assessment of the inter- and intraobserver reliability of the scoring system is

essential to ensure to work with an accurate tool.

Interobserver reliability refers to the level of agreement between different observers. Intraobserver reliability indicates the reproduce-ibility for one observer.

A reliable scoring system may be used as a basis for decision on pedicle screw revisions and may be helpful in terms of a better comparability of different studies.

Investigation of the interobserver agreement showed that revision of pedicle screws was recommended mainly for grade III screws with or without neurologic symptoms that occurred postoperatively. Grade IIIa screws should be revised due to mechanical reasons and grade IIIb screws due to a neurologic compromise.

Revision was additionally suggested for IIb screws in case of neurologic symptoms that appeared postoperatively. Similar results were obtained for the intraobserver agreement of pedicle screw revision, but calculated intraobserver agreement values were much lower for each grade. One reason might be that interpersonal discussions of the observers resulted in an increased tolerance of not optimally placed pedicle screws [(32)]. This is easy and simple we use it in this study in postoperative period to assess the accuracy of pedicle insertion of 42 screws done in current study.

Other use CT-navigation versus fluoroscopy-guided placement of pedicle screws at the thoracolumbar spine: In the lumbar spine, the placement accuracy was 96.4 % for CT-navigated screws and 93.9 % for pedicle screws placed under fluoroscopy, respectively. This difference in accuracy was statistically significant (Fishers Exact Test, p = 0.001). The difference in accuracy became more impressing in the thoracic spine, with a placement accuracy of 95.5 % in the CT-navigation group, compared to 79.0 % accuracy in the fluoroscopy group (p\0.001).

The significance of CT-navigation, especially when instrumentation of the middle and upper thoracic spine is carried out. As an alternative to other modern 3D navigation techniques, the computed tomography based navigation is an indispensable tool in these cases. In the lumbar and lower thoracic spine, both methods seem comparable.

A post-instrumentation CT scan seems to be of particular importance, allowing the surgeon to evaluate the accuracy of instrumentation before wound closure and to replace it when necessary.

Computer-assisted surgery might improve the rate of optimal pedicle screw placement [(33)]. In this study s we used screw in thoracolumbar and lower dorsal spine, and all 42 screws in scoring 1A, 1 Band B1, B2 which means correct insertion of all screw with no single revision. We have no case in our work for height or midorsal spine, which is better to use CT scan navigated screws.

The reported pedicle screw misplacement in historical spinal literature can be as high as 20-39.8%, but only a small number leads to complications neurological, vascular or visceral injuries; but these complications can be potentially life and limb threatening [(34)].

Replacement of pedicle screws should be considered and discussed depending on the radiologic findings by CT scans and the clinical aspect of the patient [(35)].

Despite reports on this accurate insertion technique, results demonstrate pedicle screw penetrations of the lateral and medial pedicle wall and pedicle screw misplacement in 4.3% [(36,37)].

Additionally, computer-assisted surgery requires a higher radiation dose and an extended operation time than do fluoroscopically controlled procedures [(38)] fluoroscopy-guided in vivo placement of pedicle screws reached a transpedicular accuracy rate of 81.6% (grade Ia) of pedicle screws.

In this study, using fluoroscopy intraoperativly only no anterior vertebral perforation was noted. In none of the patients, neurovascular complications were caused by screw placement.

In one study of accuracy of pedicle screw placement conclude that the screws positioned with free-hand technique tended to perforate the cortex medially, whereas the screws placed

with CT navigation guidance seemed to perforate more often laterally.

In conclusion, navigation does indeed exhibit higher accuracy in pedicle screw placement than free-hand technique and use of fluoroscopy even 3D scans after pedicle screw positioning cannot avoid false placement of screws and primary neurovascular damages. But screws in malposition can be detected with a high reliability.

Immediate correction of malplaced screws lowers the secondary revision rate of the patients and prevents patient's ahead secondary neurovascular problems and instability or dislocation of the fixateur [(37)].

Leakage of cerebro-spinal fluid after removal of a pedicular screw, a case of cerebrospinal fluid leakage occurring after the removal of a pedicular screw is reported. It allowed emphasizing the frequency of the dural tears in spinal surgery, particularly when pedicular screws are used. Moreover, the removal of screws having involved neurological complications can induce other lesions, such in the reported case. This removal procedure is not benign and requires precautions and a monitoring identical to the other spinal procedures [(38)].

Neurovascular risks of sacral screws with bicortical purchase, an anatomical study as a conclusion, anterior cortical penetration during sacral screw insertion carries a risk of neurovascular injury. The risk of sacral sympathetic trunk and minor vascular structures together with the major neurovascular structures and viscera should be kept in mind [(39)].

Regarding our complications in hydatid spine there is no motor and urine retention and only transit parasthesia in one case of pedicle screws, no CSF leak, and no neurovascular damage.

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Conflict of interest

There is no conflict of interest that could influence the objectivity of the research reported

Author contributions

Dr Kafaji collected part of the data and write the manuscript and Dr. Hamandi collected part of the data and designed the paper.

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Vaginal Misoprostol for Second Trimester Pregnancy Termination in Women with Prior One Caesarean Delivery

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Abstract

Background Misoprostol, a synthetic prostaglandin analogue, has become the leading mean for terminating the pregnancy. It is not clear, however, whether misoprostol is a safe abortifacient after thirteen weeks gestation in women who have a uterine scar from a previous lower segment caesarean delivery.

Objective To evaluate the efficacy and maternal side effects of misoprostol used vaginally for second trimester termination in women with a single previous lower segment caesarean delivery.

Method Sixty participants with a history of previous one lower segment caesarean delivery underwent pregnancy termination for missed abortion or lethal fetal anomaly between 14-28 weeks gestation using intra vaginal misoprostol. The dose of which was 400 microgram up to 20 weeks gestation and 200 microgram thereafter, repeated every 4 hours with a 12 hours nightly rest from misoprostol application up to a maximum of 72 hours. Women having termination for similar reasons but lacking a history of cesarean section served as a control group.

Results Abortion rate was 96.66% in the study group and 95% in the control group. The mean induction to abortion interval was 21.81±9.51 for the study group and 22.21±8.52 for the control group with no significant difference between the two groups. No cases of uterine rupture occurred in either groups.

Conclusion Inducing abortion with lower misoprostol doses appear to be safe and effective throughout the second trimester in women with a single previous lower segment cesarean delivery.

Keywords Second trimester, Misoprostol, Termination of pregnancy

Introduction

Second-trimester termination of pregnancy in women with a previous caesarean delivery is becoming increasingly common [(1)]. Although various methods for second-trimester terminationare effective, there are many risks to the patients for example: intraamniotic hypertonic saline infusion may precipitate heart failure, septic shock, water intoxication and consumptive coagulopathy [(2)].

Evacuation and curettage are associated with infection, uterine perforation and cervical trauma [(2)]. Various methods for second trimesterterminationof pregnancy have been investigated tofind the more effective methods with fewer complications to the patients.

The medical method recommended by the World Health Organization [(3)] and the Royal College of Obstetricians and Gynecologists [(4)] for termination of pregnancy between 13 and 26 weeks gestation, is the regimen of mifepristone followed by a prostaglandin analogue. When this combined regimen is used, the induction to abortion interval is significantly shorter than when prostaglandins are used alone [(3,4)].

Due to the limited access of mifepristone and greater costs of the combined method, medical abortions in the second trimester are most commonly performed by the administration of

prostaglandin analogues, using a variety of doses by various routes [5].

Induction of labor with misoprostol, a synthetic prostaglandin E1 analogue, is common practice worldwide and its use in the second trimester has shown good results [6,7,8]. The cervical ripening and uterotonic properties of misoprostol make the drug very useful [9]. Although various doses and routes of administration have been studied, the optimal dosage and route has not been defined.

Unfortunately, most of the studies have generally excluded patients with previous caesarean section. For these women, induction of labor with prostaglandins during the mid or third trimester, is considered dangerous due to the risk of uterine rupture [10,11] because of the increasing rate of caesarean deliveries which has been observed during the last two decades, the number of women with such an obstetric history who are offered pregnancy termination is also increased. The objective of our study is to assess the efficacy and safety of misoprostol used vaginally for second trimester termination in women with a single previous lower segment caesarean delivery.

Method

This prospective study was conducted between Jan. 2009 and Oct. 2010 at the Department of Obstetrics and Gynecology in Al-Kadhimyia Teaching Hospital, Baghdad, Iraq. Sixty pregnant women at 14 up to 28 weeks of gestation were enrolled. All of them had one previous lower segment cesarean delivery, and all underwent a second trimester termination for missed abortion, or lethal fetal anomaly that was confirmed by an ultrasound examination. Gestational age was calculated by the first day of a reliable last menstrual period or by a first trimester ultrasound scan if there was a discrepancy of more than 7 days.

The control group consisted of sixty women without a history of cesarean section matched for the maternal age, gestational age and gravidity to those of the study group. All patients had received authorization by the Abortion Committee of Al-Kadhimyia Teaching Hospital and all were counseled about the method for termination, side effects and possible complications. Following the counseling, a consent form was signed by all patients. Medical and obstetric history was taken and physical examination was performed. Exclusion criteria included cardiovascular or cerebrovascular disease, or a known allergy or contraindication to prostaglandins.

Additionally, women with multiple gestations, polyhydramnios or a history of myomectomy or surgery for uterine malformations were also excluded. The dose of misoprostol was 400 µg up to 20 weeks gestation and 200 µg thereafter applied vaginally every 4 hours daily, with a 12 hour nightly rest from misoprostol application, until contractions appeared but not more than 72 hours. The misoprostol tablets were placed in the posterior vaginal fornix by an obstetric resident. No additional co interventions were used.

The protocol was approved by the hospital ethics committee. Cervical progression was evaluated by vaginal examination before insertion of the next dose of misoprostol. Vital signs were monitored and the participants were checked regularly for adverse effects from misoprostol (such as fever, chills, and diarrhea) as well as signs of uterine rupture.

The occurrence of scar dehiscence or uterine rupture was assessed either clinically by observing for the signs and symptoms of dehiscence or rupture that includes: loss of uterine contractility, lower abdominal pain and tenderness at the site of the previous cesarean section scar, severe vaginal bleeding and maternal shock, or by transvaginal ultrasound examination of the site of the uterine scar.

The need for an infusion of oxytocin that was given at a dose of 10 units in 500 ml normal saline infused at a rate of 30 drops per minutes, i.e., 40 milliunit per minute, which was started at least 6 hours after the last application of misoprostol when the products of conception were retained after expulsion of the fetus; and the need for surgical evacuation which was

established after one hour of oxytocin infusion. Treatment success was defined as expulsion of the fetus within 72 hours after the initial dosage of misoprostol. Induction to abortion interval was defined as the time from the initial dosage of misoprostol to the expulsion of the fetus.

As documented at our institution, a misoprostol treatment longer than 72 hours places many women with a previous cesarean delivery at risk of scar dehiscence. This is why, on the recommendation of the institutional ethics committee, the upper limit of the interval was set at 72 hours for purposes of analysis. If expulsion had not occurred within 72 hours of the first dose of misoprostol, the participant could either receive higher doses of misoprostol or undergo pregnancy termination by hysterotomy; depending on her own wishes and the attending consultantsjudgments. Uterine curettage was performed if the placenta was not expelled within 1 hour.

Statistical analysis: performed using the x^2 test for categorical variables and the 2-tailed t test for continuous variables. Results are presented as mean, standard deviation, or as number and percentage. $P < 0.05$ was considered statistically significant.

Results

There were no significant differences in the demographic criteria of the two groups (the study and the control groups) (Table 1).

Table 1. Demographic data of the patients and the control groups

Demographic data	Study group N = 60	Control group N = 60	*P* value
Maternal age (yr)	24.8 ± 5.8	24.6 ± 6.0	0.81
Gestational age (weeks)	20.9 ± 0.72	21.5 ± 0.8	0.983
Gravidity	1.4 ± 1.28	1.43 ± 1.03	0.927

According to the dosage and time allowed by the study protocol, abortion was achieved in 58 of the 60 participants in the study group (96.66%), and in 57 of the 60 participants in the control group (95%) with no significant difference between the two groups ($P > 0.05$). There were 7 live fetuses in each group but none showed signs of life at delivery. All participants had a pretreatment Bishop score less than 4 indicating an unfavorable cervix. The mean gestational age in both groups was 21.2.

Table 2. Induction to abortion interval and total dose of misoprostol in the studied subjects

Variables		Study group N = 60	Control group N = 60	T test	*P* value
Induction to abortion interval (hours)		21.81±9.51	22.21±8.52	0.238	0.8124
TMD for gestations (µg)	< 20 weeks	720±354.6	724.13±371.9	0.061	0.9653
	≥ 20-28 weeks	592.85±224.7	614.28±204.0	0.5356	0.7584

TMD = total misoprostol dose

The mean induction to abortion interval was 21.81±9.51 hours for study group and 22.21±8.52 hours for the control group, with no significant difference between the two groups. The mean of the total misoprostol dose for those with gestations less than 20 weeks was 720±354.6 µg in the study group and 724.13±371.9 µg in the control group with no significant difference between the two groups and for gestations =or> 20 weeks up to 28 weeks, the mean dose of misoprostol was 592.85±224.7 µg in the study group and

614.28±204 µg in the control group and again with no significant difference between the two groups (Table 2).

As well there were no significant differences in the response rate to misoprostol between women aged less than 20 years compared to those aged more than 20 years in both groups (the study and the control groups) p value were 0.586 and 0.876 respectively (Table 3).

Table 3. Differences in the response rate among women aged less than 20 y and those aged more than 20 y in both groups

Group	Women responded aged ≤ 20 years N = 17	Women responded aged > 20 years N = 43	*P* value
Study group	16 (94%)	42 (97%)	0.586
Control group	16 (94%)	41 (95%)	0.876

Twenty three of the 58 (39.65%) participants in the study group and 24 of the 57 (42.105%) participants in the control group needed oxytocin infusion with no significant difference between the two groups. Among the patients who achieved induction using the study protocol, 28 participants in the study group (48.27%), and 26 in the control group (45.6%) needed surgical evacuation, and again the difference was not significant between the groups. Also there was no significant difference in the occurrence of adverse effects of misoprostol between the study and the control group. One case in the control group required blood transfusion due to occurrence of placental abruption, which causes excessive vaginal bleeding (estimated blood loss was 1250 ml). No cases of scar dehiscence or uterine rupture were observed in both groups (Table 4).

Table 4. The need for surgical evacuation, oxytocin infusion, and adverse events for those who respond to the study protocol in the two groups

Variable		Study group N = 58	Control group N = 57	X^2 test	*P* value
Surgical evacuation		28 (48%)	26 (45%)	0.082a	0.775
Oxytocin use		23 (39%)	24 (42%)	0.417a	0.518
Adverse events	Fever	30 (51%)	32 (56%)	0.226a	0.635
	Chills	11 (18%)	10 (17%)	0.039a	0.844
	Diarrhea	19 (32%)	21 (36%)	0.211a	0.646
	Ruptured uterus	0 (0%)	0 (0%)		
	Severe bleeding	0 (0%)	1 (1.7%)	1.026a	0.311
	Total	35 (60%)	34 (59%)	0.006a	0.939

About the 5 participants who did not respond to the study protocol, after counseling them, they were either offered further doses of misoprostol or undergone hysterotomy, according to their wish.

Discussion

Medical abortion was started in the late eighties, becoming more widely used in the late nineties with the mifepristone–misoprostol being widely used [12]. It came as an alternative to the dilation and curettage which resulted in many more complications [12,13]. Unfortunately, mifepristone

is not available in some countries, including Iraq. This is because of the high cost of this product and its negative connotations (it is known as an emergency contraceptive and an abortifacient, with resulting ethical dilemmas in conservative Muslim societies [14,15]

Misoprostol alone for termination of pregnancy was described for the first time in 1994. It has been used widely for termination of pregnancy in the normal uterus [12,13]. Several studies have been conducted to determine the optimal dosage and route of administration of misoprostol. Comparison between vaginal and oral administration of misoprostol for the induction of labor atterm had shown that vaginal administration was more effective, because of its pharmacokinetics [16,17]. The important feature of this study is the use of misoprostol among patients with prior one lower segment caesarean delivery undergoing second trimester abortion.

Chapman et al [18] reported a higher incidence of uterine rupture and hemorrhage with this drug than with mifepristone for women with cesarean scars, whereas others have shown it to be relatively safe [19,20,21]. These conflicting reports have led to suggest that the safety of misoprostol is yet to be established for these women [22]. The current results clearly indicate that women with previous one lower segment caesarean scar can safely terminate theirpregnancy in the second trimester by inducing vaginal birth.

Misoprostol achieved an abortion rate of 96.7%in the study group and 95% in the control group with no significant difference between the two groups ($P > 0.05$), which is in accordance with a previous study [23], reporting an 86.9% vaginal birth rate at term in women with a similar history. It is well known that the uterus becomes more responsive to uterotonic agents, and thus to lower doses of misoprostol, as gestation advances [16]. This is reflected in the present study as the total mean dose for gestations less than 20 weeks was a little bit higher than for gestations more than 20 weeks. The mean induction to abortion interval did not differ significantly between the study and the control groups.

Another important finding in the present study was that a previous one lower segment caesarean delivery does not appear to increase the incidence of complications in women who undergo a pregnancy termination in the second trimester by induction of labor. The most common maternal side effectsfound in the present study was fever followed by diarrhea and chills. However there was no significant difference in the occurrence of these minor and transient side effects between the two groups ($P = 0.9$). Placental abruption can occur at any case of abortion regardless of the agents used however, uterine rupture is the most serious complication in cases with a previous uterine scar and may occur either in the mid-trimester [24,25] or in the third trimester [26].

The incidence of uterine rupture during second trimester termination with misoprostol is unknown [21]. However, Berghella and colleagues [27] as well as GoyalV [28] in a systematic review found that the risk of uterine rupture in these women given misoprostol was only about 0.3 to 0.4 percent.Uterine rupture with the use of misoprostol has been reported more frequently in multiparous women and in women with uterine scars. Rupture is more often observed at term than in the second trimester [19].

Studies comparing the effect of misoprostol on scarred and unscarred uteri used doses higher than those used in the current study. Herabutya et al. [29] used a median total misoprostol dose of 1200 µg. One case of uterine rupture occurred, in the unscarred uterus group. Daskalakis et al [8] used a median dose of 1600 µg (range, 1200–2400 µg in their study group and 800–2400 µg in their control group). No cases of uterine rupture occurred. Daponte et al [12] used mean total doses of 655.81±109.76 µg in one group and 990±238.2 µg in another. There, too, no cases of uterine rupture occurred.

Our total mean doses in both groups (Table 1) was lower than the mean doses used in the studies just mentioned, but it was higher than that recommended in the review articles [30,31],

however, we believe the risk of a higher dose was offset by allowing an overnight rest (from 8 pm to 8 am, with a maximum of 4 doses in any 24 hour-period commencing at 8 AM). This rest, apparently, decreased the incidence of complications without increasing the rate of failure. Absence of uterine rupture in our study can be attributed to the comparatively smaller doses of misoprostol used; to the small population size and because the lower segment is not yet markedly formed and thinned out in the second trimester. The risk of uterine rupture has been reported to be higher when oxytocin is associated with prostaglandins [32]. Therefore in our study we stopped misoprostol and initiated oxytocin treatment when regular uterine contractions begun, for better titration and a lesser risk of scar dehiscence.

Overall, 47 (40.9%) of the 115 participants needed an infusion of oxytocin and 54 (46.9%) needed surgical evacuation. This could be the result of the low misoprostol dosage. Nevertheless, the protocol achieved a 96.7% rate of vaginalevacuation in the study group with no scar rupture. It is possible that higher misoprostol doses in a 24-hour period could have reduced theuse of oxytocin and the rate of surgical evacuation, but it may have also elevated the risk of scar rupture. We chose a low misoprostol dosage for the sake of safety in this prospective observational study carried out at our institute.

Bhattacharjee et al [22] used misoprostol vaginally or sublingually every 6 hours, up to a maximum of 24 hours. The dosage was 400 µg when the gestation duration was less than 20 weeks and 200 µg when it was 20 weeks or longer. The rate of protocol failure, i.e., the necessity of administering more misoprostol or performing hysterotomy or other surgical evacuation, was 27.5% in that study. No cases of uterine rupture occurred in either group. Compared to the above study the protocol set in our study had led to a much lower overall failure rate (4.16%) without increasing the rate of complications. In order to estimate the risk of complications more precisely, a very large case series is required, probably using nationally or multicentre collected data. By using national or multicentre data, confounding variables could be explored, and an exact estimate of the relative contribution to adverse outcome could be calculated.

We can conclude from this limited observational study that the prostaglandin E1 analogue misoprostol may be safe and effective at a dose of 200 µg every 4 hours in the induction of second-trimester abortion, even after the 20th week of gestation, in women with a single previous lower segment cesarean delivery.

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Conflict of interest

There is no conflict of interest that could influence the objectivity of the research reported.

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Evaluation of Ghrelin, Insulin and Leptin Levels in Obese Type 2 Diabetic Patients on Metformin or Glimepiride Therapy in Basra, Iraq

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Abstract

Background Recent evidence has demonstrated the complex function of adipose tissue and gastric cells as an endocrine organ through release of hormones into the blood stream and involved in physiological activities of the body; of them is ghrelin. The consequences of insulin resistance manifest at many levels and in many metabolic processes, producing a cluster of homeostatic abnormalities referred to what is called metabolic syndrome.

Objectives To evaluate and compare the possible effects of using metformin or glimepiride on serum concentrations of ghrelin, leptin and insulin resistance in obese type 2 diabetic patients in Basra, Iraq.

Methods Forty type 2 diabetic obese patients and twenty healthy subjects were studied. The patients were divided into 2 groups (each of 20 patients); group 1 on glimepiride therapy while group 2 on metformin treatment. Blood samples were taken after at least 8 hours fasting for measurement of serum glucose, leptin, ghrelin and insulin.

Results Ghrelin levels were significantly lower in the two patient groups with greater significant reduction in metformin group. The highest serum insulin concentration and insulin resistance levels were clearly reported in glimepiride treated group as compared to control and metformin treated group. Leptin levels show no significant differences in all studied groups.

Conclusion Metformin treatment associated significantly with improved insulin sensitivity; insulin resistance associated significantly with decreases ghrelin concentration. Ghrelin is negatively correlated with leptin and obesity while positively correlate with insulin resistance. Our data support the role of body weight as the major determinant of circulating leptin levels.

Keywords Diabetes mellitus, obesity, ghrelin, metformin, leptin.

List of abbreviations: DM = diabetes mellitus, T2DM = type 2 diabetes mellitus, IR= insulin resistance, AMPK = adenosine monophosphate-activated protein kinase, ELIZA = enzyme-linked immune sorbent assay, HOMA-IR = homeostasis model assessment-insulin resistance, CNS = central nervous system.

Introduction

Diabetes mellitus (DM) is an important public health problem with an estimated prevalence of 171 million people worldwide in the year 2000, and this number will almost double by the year of 2030 [(1)]. Type 2 DM (T2DM) is a more complex metabolic disorder characterized by obesity, impaired β-cell function, increased endogenous hepatic glucose output and insulin resistance (IR) in target tissues [(2)]. DM and obesity have a complex relationship where obesity may be a precursor for T2DM following IR [(3)]. Obesity is associated with decreased responsiveness to insulin in muscle, liver and fat. On the other hand, weight

gain associated with insulin therapy need to increase insulin dose and subsequently greater weight gain and obesity [4]. Not all subjects with T2DM are obese and many obese subjects do not have DM, but most of the subjects with T2DM are overweight or obese. These are largely preventable with change in life style and avoidance of sedentary habits and over-consumption of energy [5].

Insulin is a small protein hormone, with a molecular weight of about 6000 Daltons composed of two chains held together by disulfide bonds and synthesized in significant quantities only in β-cells in the pancreas. Binding of insulin to extracellular portion of the receptor activates its kinase activity resulting in autophosphorylation of specific intracellular tyrosine residues [6]. IR and a relative deficiency in insulin secretion contribute to the pathogenesis of T2DM [7].

Glimepiride is a potent sulfonylurea and is associated with a low rate of hypoglycemia (0.9 –1.7%). In addition to effects on pancreatic B-cell function, glimepiride also may enhance tissue sensitivity to insulin and has a favorable safety and efficacy profile with once-daily dosing of 1-8 mg/day [8]. The antidiabetic biguanide Metformin is one of the most prescribed, first-line medications in the treatment of T2DM.

In contrast to (sulphonylureas and insulin), metformin does not cause weight gain and can lead to significant weight loss [9]. One of the known targets of metformin action is the intracellular signaling enzyme, adenosine monophosphate-activated protein kinase (AMPK). In the liver and muscle, AMPK activation reduces hepatic gluconeogenesis and promotes fatty acid oxidation, respectively [10]. The consequences of insulin resistance manifest at many levels in metabolic processes, including glucose intolerance, overt hyperglycemia, hyperinsulinemia, and atherogenic dyslipidemia, collectively referred to as metabolic syndrome [11].

The role of visceral or intra-abdominal accumulation of adipose tissue seems to be strongly associated with metabolic syndrome rather than upper body subcutaneous fat [12]. Visceral adipose tissue works as an active endocrine organ able to secrete a wide variety of inflammatory cytokines and hormones with key functions in the development of DM [13].

Recent evidence has demonstrated the complex function of adipose tissue as an endocrine organ through release of hormones into the blood stream involved in physiological activities of the body with potential implication in insulin resistance, obesity and diabetes. One of the most important of these hormones is recently discovered ghrelin, which is a 28 amino acid peptide hormone, primarily produced by the stomach; it has an octanoyl group on the serine at the third position in the amino acid chain which gives the peptide hormone its biological activity [14].

Ghrelin is an appetite-stimulating hormone that increases growth hormone secretion and food intake in animals and humans [15]. The appetite stimulating effects are thought to be mediated via the arcuate nucleus of the hypothalamus and the messenger peptides neuropeptide Y and Agouti-related protein [16].

Leptin, is a single-chain proteohormone with a molecular mass of 16 kDa that is thought to play a key role in the regulation of body weight, it is produced by differentiated adipocytes, although production has been demonstrated in other tissues, such as the fundus of the stomach, skeletal muscle, liver and the placenta [17]. Leptin is a hormone that works as a mediator in the stomach – hypothalamus pathway and provides information about the body's energy storage in adipocytes in addition, its level is associated with obesity [18].

Leptin acts on the central nervous system (CNS), in particular the hypothalamus, suppressing food intake and stimulating energy expenditure [19]. Evidence suggests that circulating ghrelin may work in concert with leptin as an adiposity signal in the CNS [20]. Whether low or high concentration of such hormones is primary event in DM or secondary to anti diabetic drugs is unclear and also its relationship is ambiguous. Therefore, this study was designed to evaluate

and compare the possible effects of using metformin or glimepiride on serum concentrations of ghrelin, leptin and IR in obese T2DM patients in Basra, Iraq and to predict the relationship between above-mentioned parameters in the study groups.

Methods

Forty T2DM obese patients were attended the private medical clinic of Dr. Qais Ali Aljazaari in Basra, Iraq, from Sep. 2013 to Mar. 2014 during their periodic visit seeking for medical advice concerning their diet modification, weight reduction and drug prescription. In addition to 20 healthy subjects with age and sex matched group served as the control group.

We divided the patients into 2 groups (20 patients in each) according to their drug used to treat DM: Group 1 includes T2DM patients on glimepiride therapy while group 2 includes T2DM patients on metformin treatment for at least 4 months in both groups. The inclusion criteria were those with BMI $>$ 30 kg/m^2 and age range between 35-50 years old. The exclusion criteria includes those with BMI $<$ 30 kg/m^2, patients with chronic disease other than DM, pregnant or lactating female patients and any patient with renal or hepatic impairment, and those who are on treatment with drugs, which could interfere with the tested parameters. The study also excluded the patients with any obvious major complications of DM, including heart diseases and patients who were taking other drugs like lipid lowering medications.

Blood samples were taken after at least 8 hours of fasting in all the participants and subjects must refrained from strenuous physical activity for at least 2 hours. Serum glucose was measured by the glucose-peroxidase colorimetric enzymatic assay method. Insulin concentrations were determined by the DRG Insulin ELISA Kit, it is a solid phase ELISA based on the sandwich principle. The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on the Insulin molecule with range of assay between 1.76-100 µIU/mL.

Regarding leptin levels were measured by using The DRG Leptin ELISA Kit. The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on a Leptin molecule. An aliquot of specimen sample containing endogenous leptin is incubated in the coated well with a specific biotinylated monoclonal anti Leptin antibody.

Serum ghrelin levels were measured by enzyme Immunoassay DRG- kit were designed to detect a specific peptide (ghrelin) based on the principle of "competitive" enzyme immunoassay. The immunoplate in this kit was pre-coated with secondary antibody and the nonspecific binding sites are blocked. The secondary antibody can bind to the Fc fragment of the primary antibody (peptide antibody) whose Fab fragment will be competitively bound by both biotinylated peptide and peptide standard or targeted peptide in sample.

IR was assessed using the (HOMA-IR) according to the formula: fasting insulin (µIU/ml) x fasting glucose (mmol/l)/22.5 [21].

Statistical analysis

Values were expressed as mean ± SD; this values were statistically tested using unpaired Student's t-test and one way analysis of variance (ANOVA), supported by Bonferroni's post hoc analysis. Values with $P < 0.05$ considered significantly different. Analysis was performed using GraphPad Prism software for Windows (version 5.0, GraphPad Software, Inc., San Diego, CA).

Results

Fasting serum glucose concentrations were significantly increased in both meformin and glimepiride treated groups as compared with control but these concentrations with in upper limit of normal fasting serum glucose range (Fig. 1).

The highest serum insulin concentration and IR levels were clearly reported in glimepiride treated group as compared to control and metformin treated group this result clearly summarized in fig. 2 and 3.

Regarding leptin our finding show no significant differences in all studied groups, Obese T2DM patients on metformin therapy had higher leptin levels compared to obese control, but not significantly so as shown in fig. 4.

Ghrelin levels were significantly lower in obese T2DM patients on both metformin and glimepiride therapies as compared with control group. Ghrelin levels were significantly lower in metformin group as compared to glimepiride treated group (Fig. 5).

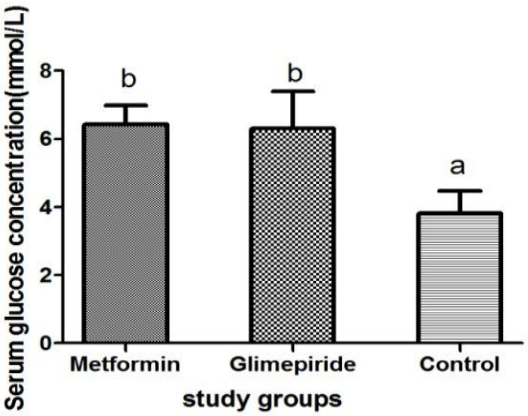

Fig. 1. Serum glucose concentration in obese type 2 diabetic patients on metformin and glimepiride therapy.

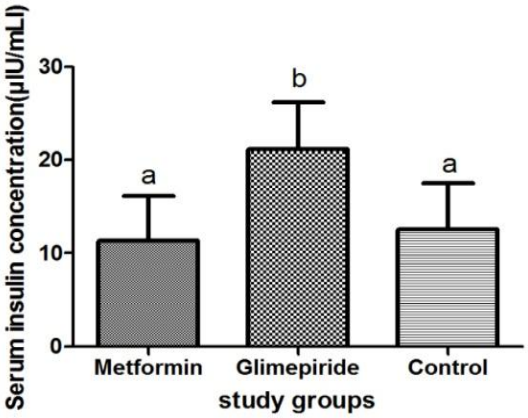

Fig. 2. Concentration of insulin in obese type 2 diabetic patients on metformin and glimepiride therapy.

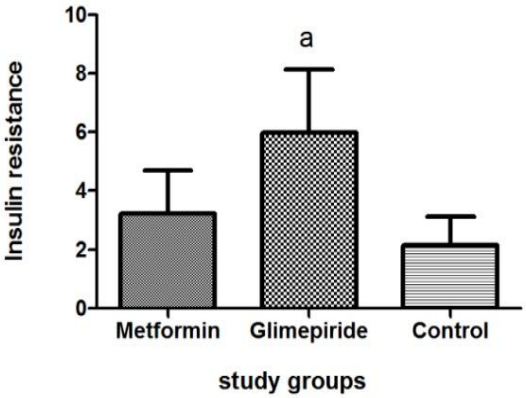

Fig. 3. Insulin resistance values in obese type 2 diabetic patients on metformin and glimepiride therapy.

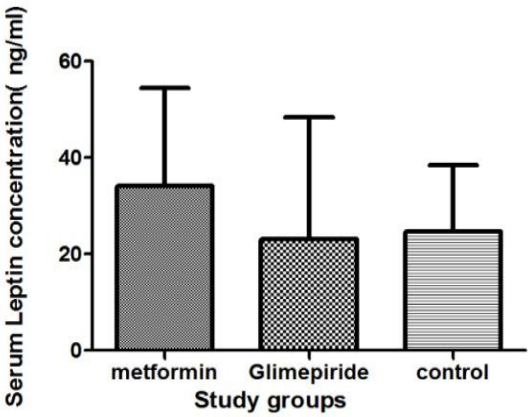

Fig. 4. Concentration of Leptin in obese type 2 diabetic patients on metformin and glimepiride therapy

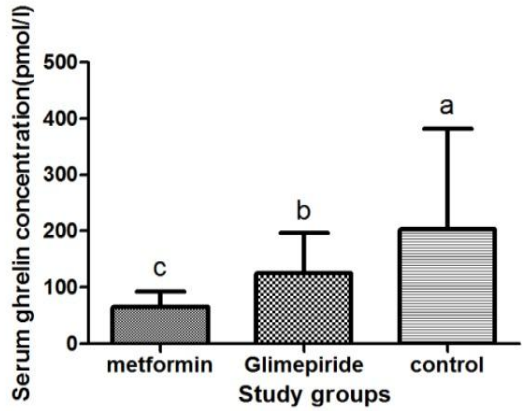

Fig. 5. Concentration of ghrelin in obese type 2 diabetic patients on metformin and glimepiride therapy.

Table 1 demonstrate the relationship between BMI, IR and studied parameters (ghrelin, leptin and insulin) the result showed there were significant correlation between IR and ghrelin, leptin and insulin in metformin treated group while the correlation observed with insulin only in glimepiride and control groups.

Table 1. Correlation between serum ghrelin, leptin and insulin levels and body mass index

Parameter	Group	Pearson Correlation Coefficient (P Value)					
		Serum ghrelin		Serum leptin		Serum insulin	
		r	*P*	*r*	*P*	*r*	*P*
BMI	**Metformin**	-0.2682	0.281	-0.1883	0.4543	-0.2895	0.2439
	glimepiride	-0.05709	0.8220	-0.1352	0.5926	0.1432	0.5709
	Control	-0.4368	0.0699	0.08646	0.7330	0.3434	0.1630
IR	**Metformin**	0.7438	0.0004*	-0.5161	0.0283*	0.9842	< 0.0001**
	Glimepiride	-0.03754	0.8824	-0.002752	0.9914	0.8782	< 0.0001**
	Control	-0.4040	0.0963	0.3080	0.2137	0.9086	< 0.0001**

IR = Insulin resistance, * = $P < 0.05$, ** = $P < 0.0001$.

In all subjects, no correlation observed between BMI and tested parameters was observed. The relationship between ghrelin, leptin and insulin among three groups are summarized in fig. 6-8. Positive correlation was observed between ghrelin and insulin in metformin treated group in other hand our finding showed negative correlation between ghrelin and leptin in control group.

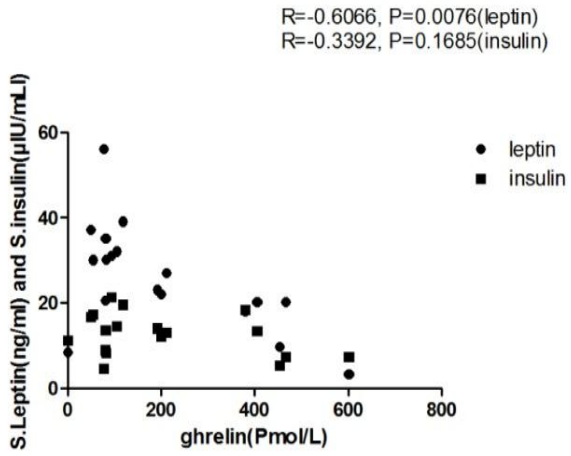

Fig. 6. Relation of ghrelin level to leptin and insulin levels in control group

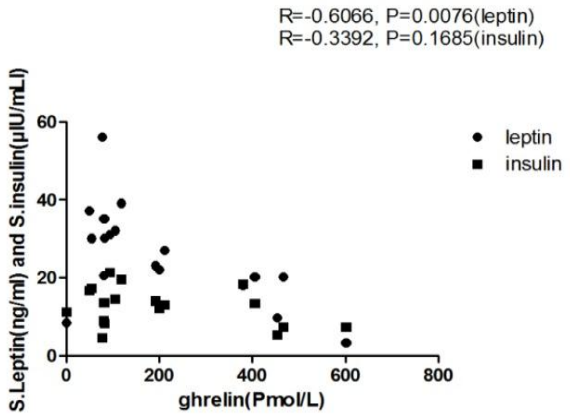

Fig. 7. Relation of ghrelin level to leptin and insulin levels in glimepiride treated group

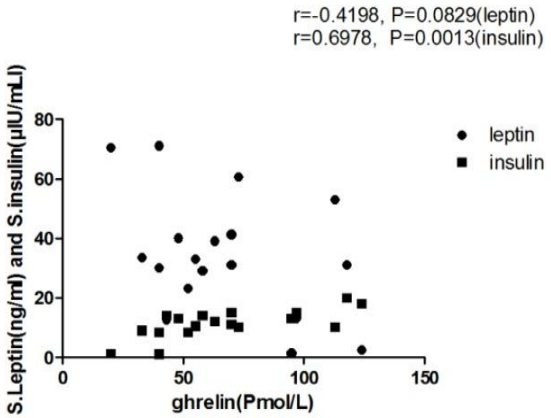

Fig. 8. Relation of ghrelin level to leptin and insulin levels in metformin treated group

Discussion

The present study demonstrated that both metformin and glimepiride treatment have beneficial effects on serum glucose concentrations in T2DM as compared with it is normal range. This result supported by Yoon *et al*. (2011), they found that glimepiride comparable to metformin in treating T2DM patients including those who are not responding well to non-glimepiride sulfonylureas [22]. Insulin resistance, their levels were significantly high in diabetic patients treated with glimepiride as compared with control and metformin treated group. This came in line with those of previous research that support the idea of greater improvement in insulin sensitivity reported in diabetes patients treated with metformin [23].

Metformin appears to have promise in obese diabetic subjects as an effective weight-loss agent with a good safety profile and has the added bonus of reducing the chronic risk factors for heart disease including hyperinsulinaemia and high low density lipoprotein cholesterol levels that are present in obese subjects [24]. In general, different mechanisms support metformin action as antidiabetic drug, the principle action is to reduce hepatic gluconeogenesis by inhibiting hepatocyte mitochondrial respiratory chain oxidation and interfere with mitochondrial energy production [25,26], possibly via an activation of the AMP-activated protein kinase [27].

The present study revealed a significant decrease in serum ghrelin concentration in all obese diabetic patients as compared with control group with significant differences was found between the three studied groups for their mean serum concentrations of ghrelin with lowest concentration observed in metformin treated group. The explanation for such finding seems to be a little bit difficult, since conflicting reports are available regarding the influence of obesity, T2DM and even antidiabetic agents on serum ghrelin concentration. Increase body weight and hyperglycemia may explain these finding. In study of Sharifi *et al*. (2013) concluded that ghrelin concentrations decreases prior to the onset of hyperglycemia and are more related to the fat pad of the body that was came in agreement of our study results [28]. Other study reported an inverse relationship between ghrelin levels, BMI and waist circumference [29]. Furthermore, plasma concentrations of ghrelin inversely associated with food intake (acute effect) and obesity (chronic effect) [30].

According to previous studies, ghrelin is one of the factors that are involved in appetite regulation [31] and acts as an appetite stimulating factor to pass starvation messages to brain. So its reduction in obesity can be considered as a defense mechanism of body to decrease appetite. Another explanation we speculate that reduction of serum ghrelin concentration in metformin treated group due to direct effect of metformin treatment on synthesis and release of ghrelin from the stomach, i.e., lead to greater reduction of serum ghrelin in metformin treated group.

A recent study by Gagnon *et al*. (2013) reported that metformin inhibits stomach proghrelin mRNA production and ghrelin secretion an effect mediated through AMPK phosphorylation [32]. The results of the present study are relatively comparable with this finding and this could explain loss of weight in people treated with metformin. One of the possible pathways by which Metformin inhibits ghrelin secretion is through AKT phosphorylation, as AMPK activation has been shown to increase AKT activity [33].

Whereas the results of this study are consistent with those of Gagnon *et al*. (2013) and another observational study [34], they contrast with the findings of Doogue *et al*. (2008), they conclude that treatment of T2DM with metformin was associated with increased plasma ghrelin concentrations, without associated changes in hunger and satiety [35].

The present study reveals no significant difference between the three study groups for their mean serum concentrations of leptin. This in line of many studies demonstrated no effect of antidiabetic drugs on serum leptin was found [36,37]. However, in other studies a decrease in

leptin levels in metformin-treated individuals has been found [38]. Several researches have been done to analyze the molecular mechanism behind the effect of metformin on leptin levels. An *in-vitro* study reports that metformin inhibits leptin secretion by inhibiting MAPK signaling pathway in adipocytes**.** Possible explanations for these discrepancies in different studies may be the length of treatment, fasting hours, BMI of studies subjects and the study population as in obese people showing a decrease in leptin levels after long-term treatment [39].

The correlation analysis of the present study showed no significant relationship between BMI and studied parameters. IR was positively correlated with serum ghrelin and insulin while negatively correlated with serum leptin with greater r-values in metformin treated group. Actually IR correlates positively with serum insulin concentrations in all study groups. These correlations can be explained, in part by the failure of beta cells to respond to the changes in glucose levels and the inability of insulin receptors to work properly to respond to insulin and this may give an indication to the roles of ghrelin and leptin levels on insulin secretion and sensitivity [40,41]. Furthermore, a significant negative correlation between ghrelin and leptin seen only in control group; this explain the effect of antidiabetic drugs on ghrelin and leptin in the present study [42]. In contrast to this idea study done by Chan *et al* reported no correlation observed between ghrelin and leptin [43].

While the treatment of hyperglycemia, has historically taken center stage in the treatment of DM, therapies directed at other coincident features, such as dyslipidemia, hypertension, hypercoagulability, obesity, ghrelin, leptin and insulin resistance, have also been a major focus of research and therapy.

In conclusion, metformin treatment was associated with significantly improved insulin sensitivity, insulin concentration and insulin resistance using (HOMA-IR) model it also reduces ghrelin concentration significantly as compared to control and glimepiride treated groups. Furthermore, the findings of the present study support that ghrelin is negatively correlated with leptin and obesity while positively correlate with insulin resistance. Since leptin levels were affected neither by metformin nor by glimepiride, our data support the role of body weight as the major determinant of circulating leptin levels.

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Author contribution

All authors contributed extensively to the work presented in this paper according to their order.

Conflict of interest

Nothing declared

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Extraction, Purification and Characterization of Lipase Produced by a Local Isolate of *Staphylococcus aureus*

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Abstract

Background *Staphylococcus aureus* is a ubiquitous bacterium that is generating increasingly bad press coverage due to its propensity to adopt a pathogenic lifestyle in hospital and community settings. Lipases catalyze both the hydrolysis and synthesis of triacylglycerols. Many of these enzymes are characterized by stability at high temperatures and in organic solvents.

Objective Purification of the enzyme by using the conventional methods and characterization of lipase.

Methods Purification included: extraction of the enzyme, the precipitation of the enzyme by ammonium sulphate, dialysis, ionic exchange chromatography by using DEAE-Cellulose (Diethylaminoethyl-Cellulose), and gel filtration by using Sephacryl S-200. Equal amounts of purified lipase solution were mixed with PBS (Phosphate buffer sodium) solutions of different pH (4,5,... until 10) and incubated in a water bath at 37 ^{o}C for 30 minutes, then the lipase activity was estimated. The purified lipase was incubated at different degrees of temperature (5, 15, ...until 85 ^{o}C) for 30 minutes. The molecular weight was determined by gel filtration chromatography.

Results The results revealed that the crude enzyme solution had a total protein concentration of 21.3 mg/ml and an enzyme activity of 257 µmole/ml. The lipase was precipitated by ammonium sulphate with 50-75%. Then the protein concentration was 4.7 mg/ml while the enzyme activity was 812 µmole/ml. Revealed that the protein concentration was 2.3 mg/ml and enzyme activity was 1020 µmole/ml. This revealed that the protein concentration was 0.9 mg/ml and the enzyme activity was 1669 µmole/ml.

Conclusion Lipase was purified to a considerable homogeneity and the characterization experiments revealed that the enzyme showed considerable heat stability and was optimally active at alkaline pH.

Key words Lipase, ion exchange chromatography, gel filtration chromatography, molecular weight.

Lists of Abbreviations: DEAE-Sephadex = Diethylaminoethyl-Sephadex, KDa = Kilo Dalton, PBS = phosphate buffer saline, SDS-PAGE = Sodium dodecyl sulfate-polyacrylamide gel electrophoreses.

Introduction

The staphylococci are gram-positive spherical cells, usually arranged in grape like irregular clusters. They grow readily on many types of media and are active metabolically, fermenting carbohydrates and producing pigments that vary from white to deep yellow. Some are members of the normal flora of the skin and mucous membranes of humans; others cause suppuration, abscess formation, a variety of pyogenic infections, and even fatal septicemia. The pathogenic staphylococci often hemolyze blood, coagulate plasma, and produce a variety of extracellular enzymes and toxins. Staphylococci rapidly develop resistance to many antimicrobial agents and present difficult therapeutic problems [(1)].

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) catalyze the hydrolysis and the synthesis of esters formed from glycerol and long-chain fatty acids. Lipases occur widely in nature, but only microbial lipases are commercially significant. The many applications of lipases

include specialty organic syntheses, hydrolysis of fats and oils, modification of fats, flavor enhancement in food processing, resolution of racemic mixtures, and chemical analyses [2].

The objective of this study was the isolation and identification of S. aureus from different body sites and lesions and studying the optimal conditions of lipase production and purification of the enzyme by using the conventional methods and characterization of lipase in relation to its stability against different temperature, pH and determination of the enzyme molecular weight.

Methods

1. Extraction and purification of lipase

Purification steps of lipase included: extraction of the enzyme, the precipitation of the enzyme by ammonium sulphate, dialysis, ionic exchange chromatography by using diethylaminoethyl (DEAE-Cellulose), and gel filtration by using Sephacryl S-200.

a. Extraction of Lipase.

Staphylococcus aureus isolate was inoculated in 100 ml of Brain heart infusion broth and incubated for 24 hours at 37 ^{o}C. Afterwards, bacterial cells were harvested by cooling centrifugation at 5000 rpm at 4 ^{o}C for 15 minutes, and then the supernatant was collected in sterile test tubes. The protein concentration was determined by Bradford [3] while the enzyme unit is defined by the amount of enzyme that catalyses the reaction of µmol of substrate per minute and measured by katal (is that catalytic activity that will raise the rate of reaction by one mole/sec in a specified assay system) that the enzyme activity was determined by Bier [4].

b. Ammonium sulphate Precipitation.

Ammonium sulphate (13.4 gm) was dissolved gradually in 100 ml of the crude enzyme extract and was mixed in an ice bath with continuous stirring for 30 minutes to reach a saturation percentage of 0-25 percent; the solution was then centrifuged at 10000 rpm under cooling condition (4 ^{o}C) for 15 minutes. The precipitate was stored in the refrigerator at 4 ^{o}C, while the supernatant was collected in sterile test tubes; the total proteins concentration and lipase activity for the supernatant were measured according to their methods.

The same process was repeated with more amount of ammonium sulphate (14.9 gm) which was dissolved gradually in 100 ml of the supernatant [of saturation percentage (0-25%)] and the other steps were completed in the same manner to reach a saturation percentage of (25-50%) then protein concentration and lipase activity were also measured as mentioned earlier.

More amount of ammonium sulphate (16.4 gm) was dissolved gradually in 100 ml of the supernatant [of saturation percentage (25-50%)] and the other steps were completed in the same manner to reach a saturation percentage of 50-75% then the enzyme activity and the total protein concentration for the supernatant were also measured as before. The supernatant (50-75%) was saturated in the same sequence to reach the final saturation percentage of (75-97%) then the enzyme activity and the total protein concentration were also measured.

The stored precipitates remnants from each step of ammonium sulphate precipitation were dissolved in a minimum amount of 0.1 M PBS pH 7, and then lipase activity and the total protein concentration were measured.

c. Dialysis.

Dialysis tube was activated by immersing in boiling distilled water (D.W.) for few minutes, the precipitate solution (50 ml) resulted from ammonium sulphate fractionation (50-75%) was poured into the dialysis tube, and then dialysis tube was incubated in a baker containing 500 ml of phosphate buffer saline 0.1 M in the refrigerator at 4 ^{o}C for 24-48 hours. The sample was concentrated by embedding the dialysis tube within sucrose powder for 30 minute.

2. Ion exchange chromatography (DEAE-Cellulose)

a. Preparation and packing of the gel

It was prepared according to Schutte [5]. Sodium azide preserved DEAE-cellulose was filtered through Buchner funnel and washed with D.W.

several times. The pH of the exchanger was adjusted to 7 by washing it several times with 0.1 M phosphate buffer saline. The ion exchanger was then degassed under vacuum, and it was ready to be added to the column.

b. Preparation of DEAE-cellulose column

It was prepared according to Schutte *et al*., [5]. A column, with a diameter of 3 cm and a length of 8cm, was washed with Phosphate buffer saline (PBS). Leaving a small amount (about 5 ml) which is enough to fill the dead space to exclude air; DEAE-cellulose gel was poured onto the column with care to avoid bubbles. The column was left overnight for packing and it was equilibrated by adjusting its pH to approximately 7 through suspending in PBS (0.1M) over night.

3. Gel filtration chromatography (Sephacryl S - 200).

a. Preparation and packing of the gel.

Sephacryl S - 200 gel was prepared according to the instructions of the manufacturing company.

4. Characterization of purified lipase.

a. Determination of optimum pH for lipase stability.

Equal amounts of purified lipase solution were mixed with PBS solutions of different pH (4, 5, 6, 7, 8, 9, and 10) all test tubes were incubated in a water bath at 37 ^{o}C for 30 minutes, then the lipase activity was estimated according to the previous method and the relationship between activity and pH of the lipase solution was sketched.

b. Determination of optimum temperature for Lipase stability.

The purified lipase was incubated at different degrees of temperature (5, 15, 25, 35, 45, 55, 65, 75, and 85 ^{o}C) for 30 minutes, then lipase activity was estimated as mentioned before and the relationship between activity and temperature was sketched.

c. Determination of the molecular weight.

Molecular weight was determined by gel filtration chromatography according to Hu and Mobley [6].

Results

1. Extraction and purification of lipase.

a. Crude enzyme.

The results of the current study revealed that the crude enzyme solution had a total protein concentration of 21.3 mg/ml and an enzyme activity of 257 µmole/ml.

b. Ammonium sulphate fractionation.

The lipase was precipitated by ammonium sulphate with 50-75% saturation percentage as a first step of purification, the results of this step revealed that the protein concentration was 4.7 mg/ml while the enzyme activity was 812 µmole/ml.

c. Dialysis.

The results indicated nearly the same value of protein concentration and the specific activity of the enzyme of the previous step.

d. Ionic exchange chromatography by using DEAE-Cellulose.

The results of the current study showed that a prominent peak of protein resulted and it was around fraction number 20-30 and was characterized by a maximal enzyme activity as shown in fig. 1. The fractions were collected, protein concentration was 2.3 mg/ml and the enzyme activity was 1020 µmole/ml. The collected fractions with higher enzyme activity were dialyzed against sucrose to reach a final volume of 5 ml.

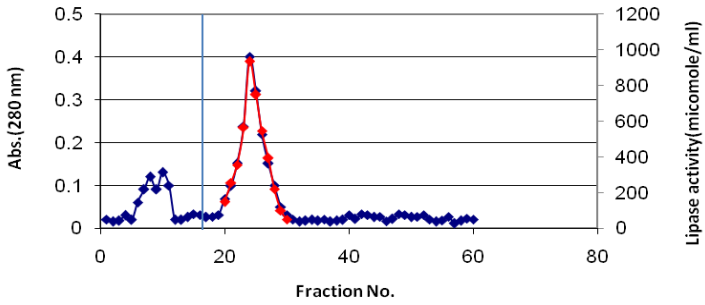

Fig. 1. Ion exchange chromatography for lipase through DEAE-Cellulosecolumn (3×8) cm. The column was calibrated with phosphate buffer saline PBS 0.1 M and pH 7.0, flow rate 40 ml/hour and 5 ml/fraction.

Table 1. Purification steps of lipase from local isolate of *Staphylococcus aureus*.

Purification step	Volume (ml)	Activity (µmole/ml)	Protein (mg/ml)	Specific activity (U/mg)	Total activity (U)	Yield (%)	Purification fold
Crude extract	100	257	21.3	12.06	25700	100	1
Ammonium sulphate precipitation (50-75%)	50	812	4.7	172.8	40600	1.6	14.3
Ion exchange using DEAE-cellulose	10	1020	2.3	443.5	10200	0.4	36.8
Gel filtration using Sephadex S-200	5	1669	0.9	1854	8345	0.3	153.7

e. Gel filtration chromatography by using Sephacryl S-200 column.

The enzyme was further purified using gel filtration and the product of dialysis (5 ml solution) was applied to Sephacryl S-200 column (Fig. 2). This demonstrated that single peak of protein was observed with a concentration of 0.9 mg/ml and the fractions number 31 through 41 correlated with that peak were collected and showed an enzyme specific activity of 1669 µmole/ml.

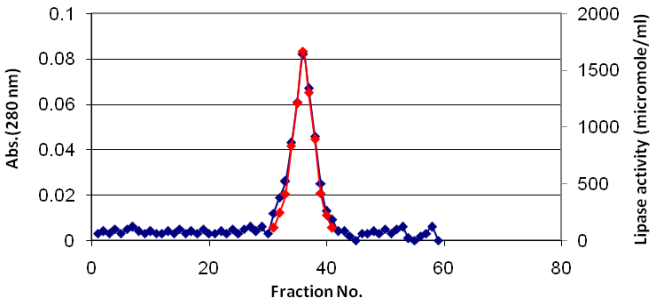

Fig. 2. The Purification of lipase by gel filtration chromatography using Sephacryl S-200 column (2x50) cm. The column calibrated with phosphate buffer saline PBS 0.1 M and pH 7.0, flow rate 60 ml/hour and 5 ml/fraction.

2. Characterization of purified lipase.

a. Determination of optimum pH for lipase stability.

For the characterization of lipase, pH dependence experiment was carried out by using several buffers with pH range from 5-10, enzyme activity was assayed. The result revealed that the activity had increased with increasing pH until pH 8; afterwards lipase activity had declined steadily (Fig. 3).

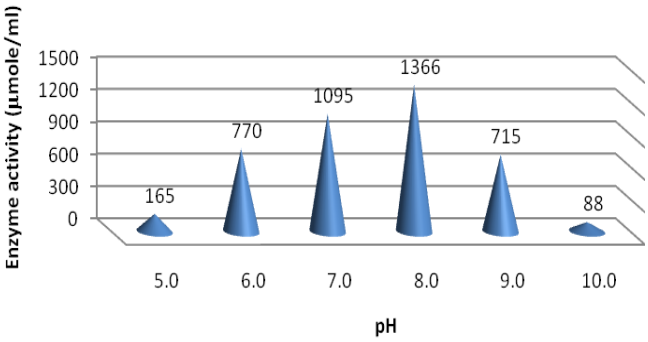

Fig. 3. Effect of different pH on lipase stability.

b. Determination of optimum temperature for lipase stability.

For determining the optimum temperature required for attaining full activity of the enzyme, temperature experiment had been performed. The purified lipase was incubated under different degrees of temperature from 10-70 ^{o}C for 30 minutes, and enzyme activity was then measured after the end of the incubation period, the result showed that lipase activity was stable as far as the temperature of incubation approached the forties Celsius but when the temperature of incubation increased, lipase activity decreased significantly (Fig. 4).

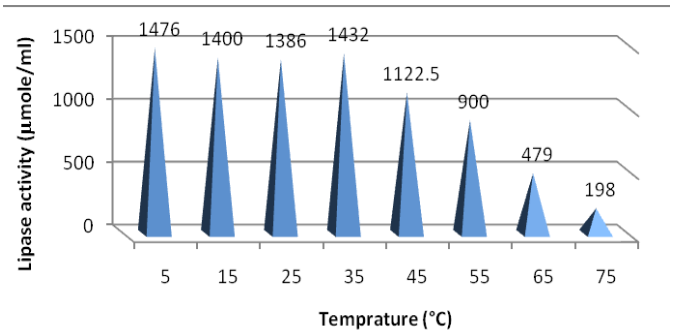

Fig. 4. Effect of different temperatures of incubation on lipase activity

c. Determination of the molecular weight of lipase using gel filtration chromotography (Sephacryl S-200).

The results of the current study revealed that when the four standards proteins of known molecular weight were eluted through the Sephacryl S-200 gel filtration column, they appeared within the elution buffer in a manner resembling their molecular weight starting from the highest to the lowest as shown in fig. 5.

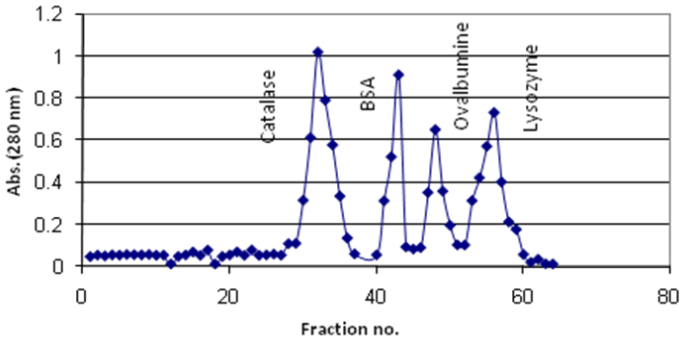

Fig. 5. Gel filtration chromatography for the four standard proteins using Sephacryl G-200 column (2x50) cm. The column calibrated with phosphate buffer saline PBS 0.1 M and pH 7.0, flow rate 60 ml/hour and 5 ml/fraction.

The construction of standard curve resembling the v_e/v_o values for the standard proteins against their relevant log molecular weight directed the way to determine the molecular weight of lipase which was about 110000 Dalton as shown in fig. 6.

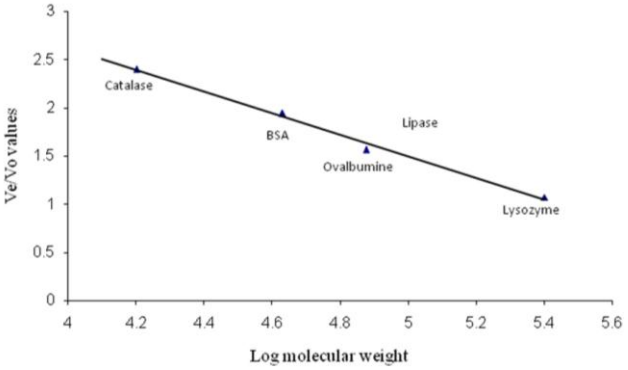

Fig. 6. Determination of the molecular weight of lipase

Discussion

In this study, the result of crude enzyme solution had a total protein concentration 21.3 mg/ml and an enzyme activity 257 µmol/ml.

In a marked disagreement with the results of the present study; Benattouche [7] found that the crude extract had a protein concentration of 67.44 mg and the lipase activity 37 unit/ml. Benjamin and Pandey [8] isolated and characterized three distinct forms of lipases from *Candida rugosa*. Three distinct forms of extra-cellular lipase were isolated by ammonium sulphate precipitation, dialysis, ultra filtration and gel filtration using Sephadex-200. The purification was 43-fold with specific activity 64.35 mg/ml.

In this study, the results revealed that the activity had increased with increasing pH until 8. Afterwards lipase activity had declined steadily.

In study of Akshalha [9], the effect of pH on lipase activity and stability were determined over a pH range of 5-9 and then residual activity was determined at pH 8.0.

The results of the present study are consistent with that of Zouaoui *et al*., [10] who stated that the pH stability of the lipase was determined by the activity retained at different pH from 3 to 10 after 1 h of incubation. The pH stability curve showed that the lipase was stable at pH 6 to 8. The stability data showed a decline in lipase activity below 6 and above 8.

Also, lipase activity was stable as far as the temperature of incubation approached the 40°C

but when the temperature of incubation increased, lipase activity decreased significantly (Fig. 4).

While Akshalha [9] mentioned that the maximum lipase activity was observed within the temperature range 30-40 ^{o}C, with different substrate, lipase from thermophilic strain *Pseudomonas putida* showed thermal stability up to 75 ^{o}C. In another study of Iftikhar *et al*., [11] the enzyme extract was incubated at different ranges of temperature and the effect of temperature on the activity of purified lipases was observed by incubation for one hour. After the end of incubation period, the result showed that lipases retained 80% of their activities at 25-30 ^{o}C and these results were nearly the same as those of the current study. Also similar to the results of the present study, the *Bacillus subtilis* lipase is most active at temperature between 30^{o}C and 50^{o}C and it retained more than 70% of its activity till 45 ^{o}C. The activity dropped rapidly above. The lipase from thermophilic *Bacilli* is relatively more stable at higher temperature. It is also mentioned that the thermal stability of lipases ranged from 20 ^{o}C to 60 ^{o}C.

The results of the current study revealed that the molecular weight of lipase obtained by gel filtration chromatography was around 110,000 dalton.

In the study of Saxena [12], lipase from strain of *S. aureus* has been purified by application of a multi-steps procedure involving ammonium sulphate precipitation and hydrophobic chromatography on phenyl-sepharose followed by gel filtration through sepharose. The molecular weight obtained by molecular sieving and electrophoresis in the presence of SDS (Sodium dodecyl sulfate) were 300 and 45 KDa. These findings are in marked disagreement with the results of the current study which may be due to application of different materials and methods for the estimation of the molecular weight. Moreover, it was found that the molecular weight of two novel lipases thermophilic *Bacillus thermocatenulatus* were of 16 and 43 KDa, respectively. The molecular weight of 16KDa is one of the smallest known of bacteria lipases [13].

In another approach to find out the approximate molecular weight of partially purified lipase, SDS-PAGE was run with protein marker. It was shown that the approximate molecular weight of the partially purified lipase is between 43 KDa and 29 KDa [14]. Other researchers found that the lipases were purified using a DEAE Sephadex A-50 column and preparative electrophoresis and purified enzyme from *A. repens and Eurotriumhebariorum* had molecular masses of 38 and 65 KDa, respectively as determined by SDS-BAGE [15].

In conclusion, *staphylococcus aureus* was isolated as one of the important causative agents of multiple body site infections. Lipase production was affected by incubation conditions and constituents of culture media indicating that this enzyme can be used by the bacteria in a versatile manner for the establishment of infection in various body sites. Traditional methods of purification can be of value for studying bacterial virulence factors and/or other products as those methods yielded results comparable to other studies that utilized different purification strategies and methods. It is evident that lipases have a wide practical application industry and medicine and are available with abroad range of properties depending on their source. With a molecular weight exceeding 100 kDa, it appeared that staphylococcal lipase is one of the biggest lipases that have been purified.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

Asia Fadhil collected the sampling and analysis and discus the result and Dr. Amer Hani supervised the research.

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Relation of Antimüllerian, Follicular Stimulating Hormone and Antral Follicle Count on Intracytoplasmic Sperm Injection Outcome in Infertile Patients

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Abstract

Background Studying some of fertility-related hormones is of major benefit to identify the causative factors and to search for an appropriate treatment. Anti-müllerian hormone regarded as quantitative markers for ovarian reserve. Basal follicular stimulating hormone provides a picture of how well the hypothalamic-pituitary-gonadal axis is functioning and is the most commonly used tests for predicting success in intracytoplasmic sperm injection (ICSI) treatment.

Objective To evaluate the level of serum and follicular fluid antimüllerian hormone, serum follicular stimulating hormone and antral follicle count and its relation to ICSI outcome in infertile patients.

Methods Seventy four infertile women were selected randomly from those attending the Fertility Centre, Al-Sader Teaching Hospital, Al-Najaf /Iraq. Ultrasound was performed for antral follicle count and their measurement at cycle day 2. Hormonal analysis is done for serum follicular stimulating hormone at cycle day 2 and for serum and follicle fluid antimüllerian hormone at day of ovum pickup.

Result The fertilization rate was positively correlated with follicular fluid antimüllerian hormone (r = 0.303; P = 0.048) but not with serum follicular stimulating hormone, serum antimüllerian hormone and antral follicle count.

Conclusion Follicular fluid antimüllerian hormone level was positively correlated with fertilization rate, while serum antimüllerian hormone level does not affect the fertilization rate in ICSI cycle. The basal follicular stimulating hormone level do not relate to fertilization rate, and the same thing regarding antral follicle count.

Keywords Anti-müllerian hormone, follicular stimulating hormone, antral follicle count, intracytoplasmic sperm injection.

List of Abbreviation: AMH = antimüllerian hormone, FSH = follicle stimulating hormone, AFC = antral follicle count, CD2 = cycle day two, FR = fertilization rate, ICSI = intracytoplasmic sperm injection, ART = Assisted reproductive technology, IVF = in vitro fertilization, OR = ovarian reserve, 3D = three dimension, 2D = two dimension.

Introduction

Infertility affects approximately 13-14% of reproductive-aged couples and it might be due to ovulatory disorder in 27%, abnormal semen in 25%, tubal occlusion in 22%, endometriosis in 5% and unexplained in 21% of cases [1,2].

Intracytoplasmic sperm injection (ICSI) is an assisted reproductive technology (ART) used to treat sperm-related infertility problems. Many fertility programs routinely do ICSI on some of the eggs even if everything is normal [3].

The use of early follicular-phase follicular stimulating hormone (FSH) as a marker of ovarian reserve (OR) and fertility outcome was proposed many years ago, where basal FSH levels were predictive of estradiol response, oocyte yield, and pregnancy rates [4].

The earliest and most consistent reproductive endocrine finding associated with reproductive aging in women is a "monotropic" elevation in FSH, which reflects poor hormone production from an aging pool of ovarian follicles and disinhibition of FSH production [(5)]. Since FSH levels are co-regulated by inhibin, it has been suggested that decreased secretion of ovarian inhibin by the decreasing follicular pool, may be primarily responsible for the monotropic rise in FSH [(6)]. Currently, women with raised FSH levels (> 10 IU/ml) in the early follicular phase are counseled against in vitro fertilization (IVF) treatment due to their probable poor response to stimulation [(6)].

Basal FSH, through the feedback of inhibin B and estradiol, will represent cohort size but mostly at the extremes and therefore give a more thorough indication of quality aspects. This is in contrast to the more direct quantitative tests using antral follicle count (AFC), antimüllerian hormone (AMH) and ovarian volume that are capable of describing a more complete range of OR states [(7,8)].

AMH level is not affected by classical endocrine fluctuations of the menstrual cycle, and it plays a role in the regulation of ovarian function during both early and late follicle development. It can be considered a factor that reflects the depletion rate of the primordial follicle pool and affects the maintenance of the pool of growing follicles [(9,10)]. Women with higher AMH values will tend to have better response to ovarian stimulation for ICSI; more eggs retrieved and give a higher success rate [(11)].

The objective of this study is to evaluate the level of serum and follicular fluid AMH, serum FSH and antral follicle count and its relation to ICSI outcome in infertile patients.

Methods

A hospital-based cohort study was conducted to determine the levels of AMH, FSH and AFC with fertilization rate in ICSI cycle. Seventy four infertile women agreed to participate in this research were selected randomly from those attending the Fertility Centre, Al-Sader Teaching Hospital, Al-Najaf Holly City during the period from March 2013 to November 2013. The study was approved by the Institute Review Board of the College of Medicine, Al-Nahrain University.

The mean age for infertile female patients was 31.41±5.45years. Infertility due to a female cause was present in 39 (52.7%) and to a male cause in 35 (47.3%) of the cases. Primary infertility was present in 57 (77%) whereas only 17 (23%) patients have secondary infertility. The duration of infertility for the entire patients group (8.11±3.94 years).

Full history and general examination, pelvic examination and transvaginal ultrasound examination at cycle day two (CD2) were performed for each infertile women for antral follicle count measurement. Those women with visible ovaries on ultrasound, no uterine fibroid, uterine anomaly or ovarian cyst measuring ≥20 mm in diameter, negative screening tests for hepatitis B and C, as well as for human immune deficiency viruses, inability to achieve pregnancy in a period of ≥ 12 months despite regular unprotected intercourse, no history of heart, liver, or kidney disease and no matter the cause of infertility was female or male factor were selected for the study.

Once the couple has been selected according to our selection criteria, they were randomized to go on with the designed program. The dependent variable for this study was the FR. The independent variables of this study were the FSH, AMH and antral follicular counts. All the participants were asked to come back on CD2 for complete medical evaluation. They were subjected to the routine steps of infertility assessment, usually performed by their own primary physician, to evaluate their fitness for the ICSI program; by the following:

Transvaginal sonography: performed by a specialist using ultrasound device with a vaginal probe (5-7 MHZ) aiming for antral follicle count and their measurement.

Hormonal analysis: Ten ml of venous blood samples were collected in plain tubes at CD 2 between 08:00-10:00 am and left at least 15 minutes at room temperature before

centrifugation at 3000 rpm for 10 minutes. Serum aliquots were obtained to measure FSH by miniVIDAS technique 2-3 hours following blood aspiration.

Another blood and follicular fluid samples were taken from the patients at the day of ovum pick up for later measurement of AMH by sensitive Enzyme Linked ImmunoSorbant Assay technique (ELISA).Under general or local anesthesia, ovum pick up through transvaginal aspiration usually timed 34-36 hours following human chorionic gonadotrophine injection and carried out via ultrasound guidance. Those patients eligible for ICSI cycle were scheduled for oocyte pick up after programmed ovulation induction.

During the ICSI procedure, the head of a single sperm is injected into the egg, eliminating the need of the sperm to penetrate the egg for fertilization. A full ICSI cycle includes a number of steps

Step 1: Ovulation stimulation and egg retrieval

Step 2: Sperm retrieval

Step 3: Fertilization

Step 4: Embryo transfer

To check for fertilization of oocytes, the fertilized oocytes must be examined 16-20 hours after insemination for the presence of two round nuclear structures, the male and female pronuclei (PN). The cells surrounding the eggs are carefully dissected away to allow clear visualization of the egg. Pronuclei must be scored within the appropriate time span, before they merge and are no longer visible. This ensures only normal zygotes with two pronuclei (2PN's) are cultured for embryo transfer.

Data Analysis

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 18. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as mean and standard deviation 95% confidence interval. Pearson's correlation coefficient was used to compare between two continuous variables. A *P* value of ≤ 0.05 was considered as significant [12].

Results

Table 1 shows that the majority of infertile patients had FSH level < 9 mIU/mL, serum AMH level > 1 ng/ml, and follicular fluid AMH level > 1 ng/ml. Similarly, the AFC was < 10 and FR was ≥ 50 % in the majority of our patients (Table 1).

Table 1. Illustrates the hormonal status, antral follicular counts and fertilization rate of infertile patients

Reproductive Parameters		(%)	Mean ± SD
FSH	< 9 mIU/mL ≥ 9 mIU/mL	97.3 2.7	4.40±2.23
Serum AMH	≤ 1 ng/ml > 1 ng/ml	20.9 79.1	1.88± 1.17
Follicular Fluid AMH	≤ 1 ng/ml > 1 ng/ml	23.3 76.7	2.06 ± 1.51
Antral follicular count	≥ 10 < 10	39.2 60.8	11.24 ±5.13
Fertilization rate	≥ 50 % < 50%	64.9 35.1	0.61 ± 0.30

FSH = follicular stimulating hormone, AMH = antimüllerian hormone, Level of FSH and AMH represent the cutoff levels of those hormones according to the kit used. Antral follicle count:10 represent median number of all follicle counted. Fertilization rate:50% represent median number of fertilization rate.

The fertilization rate was positively correlated with follicular fluid AMH hormone (r = 0.303; P = 0.048) (Fig. 1) but not with serum FSH, serum AMH and AFC (Table 2).

Table 2. Correlation of fertilization rate with antral follicle count, serum FSH, serum AMH and follicular fluid AMH hormone levels

	Variable	Mean± S.D	*r*	*P* value
Fertilization rate	AFC	11.24 ± 5.13	0.063	0.596
	FSH (mIU/ml)	4.40 ± 2.23	-0.032	0.788
	Serum AMH (ng/ml)	1.88 ± 1.17	0.103	0.512
	FF AMH (ng/ml)	2.06 ± 1.51	0.303	0.048*

FSH = Follicular Stimulating hormone, AMH = antimüllerian hormone, FF = follicular fluid, AFC = antral follicle count.

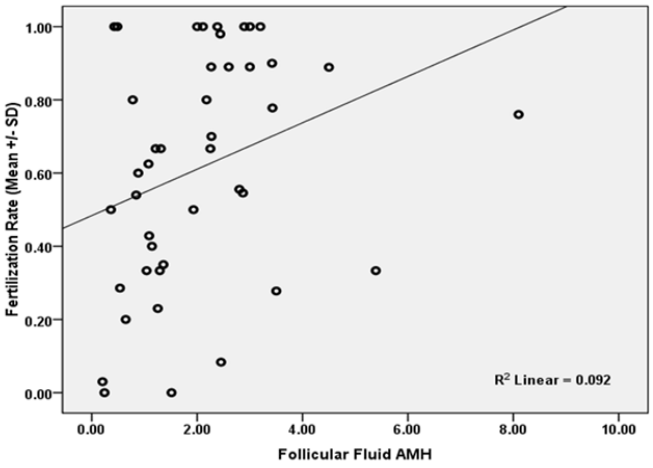

Fig.1. Correlation of fertilization rate with follicular fluid AMH

Discussion

The AMH level in serum and follicular fluid of the infertile women lies within the normal standard levels and were comparable to values noticed by Lee *et al* and Freour *et al* who demonstrated follicular fluid AMH level to be approximately 1 ng/ml [13,14]. Low ICSI outcome and poor response reported to be associated with serum AMH levels < 1 ng/mL, normal response with levels from 1-4 ng/mL, and high response with levels > 4 ng/mL [15-17].

In the present study, follicular fluid AMH hormone level was positively correlated with FR. This influence could be related to that AMH is produced in females by the granulosa cells from the pre-antral and antral follicles in the human fetus after 36 weeks of gestation [18]. The levels of AMH reflect the number of preantral follicles and thus are a marker of oocyte pool - a germinal reserve of the ovary for reproduction.

AMH also has a direct autocrine-paracrine effect on the granulosa cells, oocyte function and embryo quality. It seems to be a promising parameter for early detection of reduced OR as well as ovarian dysfunction, thus AMH, which indicate ovarian aging and OR can become a critical factor in infertility [19]. Several studies have demonstrated that AMH is a better marker of OR than age, basal FSH, estradiol and inhibin [20].

Furthermore, basal serum AMH levels <1.1 ng/ml were associated with IVF failure and this finding support that AMH levels are believed to be a reflection of the number of growing follicles, which is also related to the number of small antral follicles [21].

Likewise, AMH levels have also been shown to be 10-fold lower in the cancelled cycles compared with patients who had a complete IVF cycle. In ~75% of cancelled cycles, AMH levels were below the detection limit (0.098 ng/ml) [22]. Besides women with higher AMH values tend to have better response to ovarian stimulation for IVF/ICSI, have more eggs retrieved, gives a higher fertilization and pregnancy rate [14].

The data of the present study strongly support the previously published reports dealing with the prognostic value of the AMH on its relation to fertility and ICSI outcome.

The basal FSH level in the current study was comparable to that reported by Vladimirov *et al* and Göksedef *et al* [23,24]. Our study showed that the basal FSH do not relate to FR.

FSH levels vary during the menstrual cycle and will peak prior to ovulation. FSH blood tests are generally performed on the second or third day of menstrual cycle.

Basal FSH levels provides a picture of how well the hypothalamic-pituitary-gonadal axis is functioning [25], as it measures pituitary production of FSH in response to feedback from ovarian hormones and it is the most commonly used tests for predicting success in ICSI treatment.

A normal FSH level probably indicates a good OR. However, elevated FSH levels may suggest impaired OR [23].

The result of the present study was in accordance with the findings of Smotrich *et al* and Evers *et al* who declared that normal FSH, have been associated with improved stimulation response, higher correlation with fertilization and hence pregnancy rates and lower cycle cancellation rates [26,27].

The AFC in the present study does not correlate with FR, which disagrees with the finding of Muttukrishna *et al* that demonstrate clear association between AFC and the number of eggs collected and the likelihood of good fertilization and clinical pregnancy [28]. This discrepancy might be due to the sample size being smaller in the current study, or to the stimulation protocol used in the Fertility Center; since the antagonist analogue have not been introduced yet, rather short or long protocol was used. Alternatively this difference could be related to the limited types of gonadotropins accessible in our country.

In conclusion, Follicular fluid AMH hormone level was positively correlated with FR while serum AMH, basal FSH and antral follicle count do not relate to FR.

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Author Contribution

The authors share the responsibility in preparing and completing this work.

Conflict of interest

The authors declare no conflict of interest.

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Evaluation of Serum and Urinary Fibronectin as a Diagnostic Marker of Bladder Cancer

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Abstract

Background Accurate and sensitive detection of bladder cancer is important to diagnose this deadly disease at an early stage, estimate prognosis, prediction the response to treatment and for monitoring the recurrence. In past few years, laboratory diagnosis and surveillance of urinary bladder cancer have improved significantly. Although, urine cytology remains the gold standard test, many new urinary biochemical markers have been identified.

Objectives To evaluate the value of fibronectin in serum and urine to detect bladder cancer in different grades and stages.

Methods Thirty five patients diagnosed as bladder cancer with mean age 61.94±11.66 years and thirty five aged-matched healthy volunteers as control group were included in this study. Serum and urinary fibronectin were measured by ELISA technique.

Results The mean±SEM serum and urine levels of fibronectin in patients with bladder cancer (33.11±1.90 µg/ml; 33.08±1.12 ng/ml respectively) were significantly higher than the levels in control group (8.57±1.10 µg/ml; 7.58±1.00 ng/ml, respectively). When using a serum fibronectin concentration of 25.65 µg/ml as a cutoff value for the diagnosis of bladder cancer, sensitivity was 71.4%, specificity 100%, the positive predictive value was 100% and the negative predictive value 77.78%, and the sensitivity and specificity of urine fibronectin were (94.3%, 97.1% respectively); when using a urine fibronectin concentration of 20.00 ng/ml as a cutoff value for the diagnosis of bladder cancer. The positive predictive value was 97.05%, and the negative predictive value was 94.44%.

Conclusion The measurement of fibronectin level in serum and urine is useful in discriminating bladder cancer patients from normal subjects.

Key words Serum and urinary Fibronectin, Bladder cancer.

List of abbreviation: FN= Fibronectin, KD= Kilo Dalton, ECM= Extracellular matrix, BC= Bladder cancer, ROC = Receiver operator characteristic.

Introduction

Bladder cancer (BC) is a major health problem across the world, mainly due to its association with tobacco abuse. The final diagnosis is achieved through cystoscopy and resection of tumors for pathological examination [(1-3)]. It is the fourth most common cancer in men in the USA and the eight most common cancers in women, with an estimated 57,400 cases being diagnosed in 2003, resulting in 25,100 deaths. Commonly accepted risk factors for Transitional cell carcinoma of the bladder include cigarette smoking, occupational exposure to aniline dyes, benzidene compounds, analgesic abuse (phenacetin) and chronic irritation such as indwelling catheters. Most cases of bladder cancer are superficial at the time of diagnosis (stage Ta- T1). The recurrence rate of superficial tumors can be as high as 70 % with 10- 15 %

progressing to muscle invasive disease. The risk of progression is directly related to tumor grade and stage [4].

Bladder malignancies can be treated using different approaches such as transurethral resection for superficial tumors, intravesical chemotherapy and radical cystectomy for non-metastasized tumors, or by systemic chemotherapy for locally advanced or metastasized tumors [5].

Cytological diagnosis is noninvasive and has high specificity but low sensitivity, especially for low-grade tumors. At the same time, it can be a challenging test to perform and highly dependent on the skills and experience of a trained cytopathologist. Because cystoscopy is invasive procedure and because cytology has poor sensitivity, non invasive biomarkers have been sought as alternatives to cystoscopy and cytology for the detection and surveillance of bladder cancer [6].

Fibronectin (FN) is one of the extracellular matrix member that is found in the urine of normal individuals, but found in a higher amount in patients with bladder cancer [7,8]. It is 440-KD glycoproteins as a well characterized extra cellular matrix (ECM) protein playing an important role in the inhibition of cellular attachment and tumor spread. The mechanism of FN action is mediated by specific receptors and growth factor [9-11].

The FN molecule appears to be important in wound healing, is found at sites of inflammation, and functions in normal cell-to-cell cohesiveness [12]. In the urinary tract, FN has been localized to the urothelial basement membrane [13].

Fibronectin is synthesized by many cell types [14]. A large portion of circulating fibronectin is produced by hepatocytes, in which it exists in two forms, termed cellular fibronectin (cFN) and plasma fibronectin (pFN) [14]. Plasma fibronectin is a soluble form produced solely by hepatocytes, whereas cellular fibronectin is an insoluble form produced by a variety of cells and incorporated into tissue extracellular matrix. Both isoforms are generated from a single gene by alternative splicing [15]. In healthy subjects, the human plasma fibronectin level is 300±100 µg/ mL [16], with no differences according to gender or age [17].

The present work was aimed to measure serum and urinary fibronectin in bladder cancer patients.

Methods

Thirty five patients with bladder cancer with mean age 61.94±11.66 years and 35 age-matched healthy subjects (controls) with mean age of 59.54±10.18 years were studied. Blood and urine samples from all 70 subjects were collected from Al-Imamain Al-Kadhimain Medical City, Baghdad, Iraq, between September 2012 and August 2013. The approval of the Al-Nahrain University/ college of Medicine Research Ethics Committee and written consent of every patient included in the study were obtained. All control subjects were with different non malignant urological disorders (i.e., hydrocele testis, ureteropelvic junction obstruction, stone disease, urinary incontinence). Patients with other malignancies in their medical history were excluded.

Diagnosis of bladder cancer was based on clinical assessment; cystoscopy was done for all patients as the reference standard for identification of bladder cancer. All tumors and suspicious lesions found were either resected or biopsied. The final diagnosis of bladder cancer based on histopathological examination. Fresh voided urine samples and blood were collected from 35 patients with newly diagnosed bladder cancer before they underwent transurethral resection of bladder tumor (TURB). Additionally urine and blood samples were collected from 35 healthy volunteers (controls). Blood samples were centrifuged at 3000 rpm and serum was stored at -40 ^{o}C, urine samples were centrifuged at 3000 rpm and the supernatant was pipette and stored at -40^{o}C. Urothelial cancer grading and staging were performed according to the World Health Organization criteria [18]. Serum and urine fibronectin levels were measured by monoclonal antibody Enzyme Linked Immuno Sorbent Assay (ELISA) technique.

The values of laboratory tests are presented as mean ± standard deviation and mean ± standard error for mean (SEM). The comparison of means between the different groups was performed using the Student's t test.

Receiver Operator Characteristic (ROC) curves was constructed to plot sensitivity against specificity of high serum, urine fibronectin levels as diagnostic tests for BC. The areas under the ROC curves (AUC) were calculated and compared with the AUC (0.5) of the non-diagnostic test (the line with the slope). To determine the cut-off values of significant sensitivity and specificity (>70%); contingency tables (cross-tabs) were constructed for the calculation of positive and negative predictive value were calculated.

All other analyses were performed using SPSS version 16 computer software (Statistical Package for Social Sciences). A *P* value less than the 0.05 level of significance was considered statistically significant.

Results

The concentration of serum, urine fibronectin of the studied subjects is summarized in Table 1. Serum fibronectin levels were significantly higher in the patients group with BC compared with the controls ($P < 0.001$).

Table 1. Serum and urine fibronectin levels of the subjects studied.

Parameter		Controls N = 35 mean±SEM	BC N = 35 mean±SEM
FN	Serum µg/ml	8.57±1.10	33.11±1.90*
	Urine ng/ml	7.58±1.00	33.08±1.12*

BC = bladder cancer, * $P = 0.001$

The ROC curves demonstrated a significant discriminatory ability of increase serum FN levels for the diagnosis of bladder cancer. The AUC for serum FN was 0.966 (95% CI: 0.931-1.001). A significant difference was found in the BC patients group ($P < 0.001$) as seen in fig. 1 and table 2.

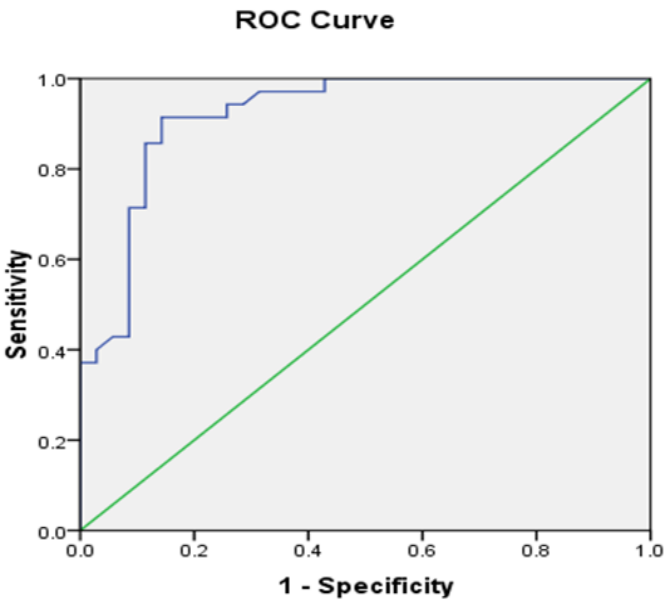

Fig. 1. Receiver Operator Characteristic (ROC) curve of high serum FN levels as a diagnostic test for bladder cancer

Table 2. Area under the curve for ROC analysis of parameters with testing for statistical differences

Fibronectin	AUC±SEM	95% CI
Serum	0.966±0.018	0.931-1.001
Urine	0.976±0.020	0.937-1.016

$P = < 0.001$

When the serum FN concentration of 25.65 µg/ml was used as a cutoff value for the diagnosis of bladder cancer in the control group; the sensitivity was 71.4%, specificity was 100%, and the positive predictive value was 100%, while the negative predictive value 77.78% (Table 3).

Table 3. Validity Indications of serum and urine fibronectin levels in prediction of bladder cancer

Parameter	Fibronectin	
	Serum	Urine
Cutoff value	25.65µg/ml	20.00ng/ml
Sensitivity	71.4%	94.3%
Specificity	100%	97.1%
PPV	100%	97.05%
NPV	77.78%	94.44%
Accuracy	85.71%	95.71%

Urine FN levels were significantly higher in patients with BC compared to the control group (p< 0.001) as shown in table 1. The receiver operating characteristic analyses showed that urine FN values can be used for the diagnosis of BC from the control group, with the areas under the curve being 0.976 (95% CI: 0.937-1.016). A significant difference was found in BC ($P < 0.001$) as noticed in Fig. 2 and table 2.

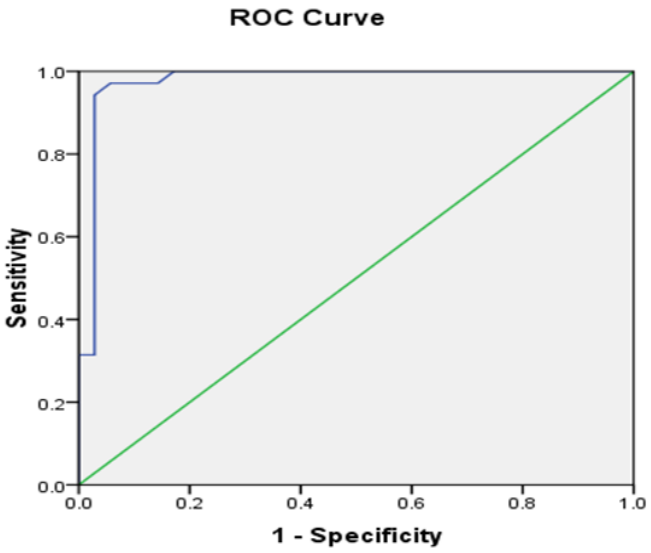

Fig. 2. Receiver Operator Characteristic curves of high urine fibronectin levels as diagnostic tests for bladder cancer from control group.

When using urine FN concentration of 20.00 ng/ml as a cutoff value for the diagnosis of bladder cancer from control group, sensitivity was 94.3%, specificity 97.1%, the positive predictive value was 97.05% and the negative predictive value 94.44% (Table 3).

Discussion

Development of new methods for bladder cancer detection is required because cystoscopy is invasive, and voided urine cytology has low sensitivity. The goal of the present study was to evaluate the clinical suitability of promising bladder tumor marker, named fibronectin in serum and urine. The high serum FN level in bladder cancer was explained by the formation of metastases and local progressions of tumors presuppose degradation of extracellular matrix (ECM) components. These essential steps are possibly mediated by hydrolytic enzymes of the tumor itself and induced by the tumor in the stromal cells [9,19]. Products of this various ECM components degradation are released in the circulation and determination of these components can be helpful for early detection of several malignancies [20].

The results of the current study are similar with the study by Hegele *et al*. who found that patients suffering from bladder cancer showed significantly higher serum FN levels. It was found that the mean serum fibronectin in the cancer group was significantly higher compared to the control group ($P < 0.001$); this result is in agreement with the results obtained by (Kirkali *et al*., Who found a significant elevation of fibronectin level in tissue of bladder cancer patients [21].

It is very interesting that, by means of ROC curve analysis (Fig.1), the measurement of serum FN level was found to be a reliable test for discriminating bladder cancer from normal subjects, when the positive predictive value (PPV) was interestingly high (100%) and the negative predictive value (NPV) for the diagnosis capacity in the exclusion of bladder cancer versus normal subjects was accepted (77.78%). The sensitivity and specificity were (71.4% and 100% respectively).

From these findings, it can be stated that the test is quite specific to differentiate the normal and bladder cancer subjects.

Urine fibronectin mainly originates from the basement membrane of the bladder, not from the kidney, since renal glomeruli cannot filter this large protein to the urine. Turnover of the suburothelial matrix can be responsible for the low but measurable urine FN level in healthy individuals [22,23].We evaluate the use of urinary fibronectin as a tumor marker of bladder cancer.

In this study, it was found that urine fibronectin level to be significantly higher in bladder cancer group ($P < 0.001$) than in normal group.

So it was concluded that the urine fibronectin measurement is useful to differentiate normal subjects from subjects with bladder cancer. This

result is consistent with the results of other studies [8,23-25]. Although each author found its own cutoff value, most of them conclude that urine Fibronectin measurement is important to discriminate bladder cancer subjects from normal subjects.

Criteria for the ideal tumor marker, has been described by Huben [26]. Urinary fibronectin fulfills most of these criteria. It is easy to do, relatively inexpensive, found in body fluid that iseasily collected, and not affected by other variables like systemic diseases [27].

In conclusion, measurement of serum fibronectin may be of value in the early diagnosis of bladder cancer. Urine FN test has a very good accuracy (95.71%) by the test of ROC analysis, when used to differentiate between bladder cancer and normal subjects.

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Author contribution

Dr. Abdul-Rasheed suggests the study and co-writes the manuscript; Dr. Al-Nasiri makes the diagnosis of patients and Miss. Habash writes the paper and analyzed the results statistically.

Conflict of interest

There was no conflict of interest.

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Omentin-1 Level in Middle Age Women with Hypothyroidism and their Relations to Risk factors of Cardiovascular Disease

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Abstract

Background Omentin-1 is fat deposition-specific adipokine that is highly and selectively expressed in visceral adipose tissue. Low circulating levels of Omentin-1 have been associated with endothelial dysfunction and cardiovascular disease. Abnormalities of lipid metabolism associated with subclinical and overt hypothyroidisms patients, low Omentin-1 level can affect risk factors for cardiovascular disease.

Objective To determine the differences in the levels of Omentin-1 in middle age women with subclinical and overt hypothyroidisms and correlate its level with parameters which considered risk factor for cardiovascular disease.

Methods Ninety middle age women divided into three groups as follows: group I consisted of 30 healthy women as a control subject, group II comprised 30 women with subclinical hypothyroidisms, group III include 30 women with overt hypothyroidisms, serum of Omentin-1, high sensitive c-reactive protein and lipid profile levels were evaluated in patients and control groups.

Results Serum omentin-1 levels were significant decreased in patients with subclinical and overt hypothyroidisms compared with control group. Significant negative correlation between omentin-1 and thyroid-stimulating hormone, high sensitive c-reactive protein, total cholesterol, atherogenic index in patients was found. Significant positive correlation was observed between Omentin-1 and high-density lipoprotein in the patients.

Conclusion we conclude that serum Omentin-1 levels were decrease in middle age women with hypothyroidism and its correlate with altering lipid profile, high levels of atherogenic index and high sensitive c-reactive protein, all of these conditions is correlated with cardiovascular disease, so Omentin-1 in hypothyroidism patients is a risk factor for cardiovascular disease, our suggestion that possible follow up serum lipid profile and omentin-1 monthly for middle age women to prevent cardiovascular disease.

Keywords Omentin-1, lipid profile, hypothyroidism disease

List of abbreviation: SHT = subclinical hypothyroidisms, OHT = overt hypothyroidisms, CVD = cardiovascular disease, OM-1 = omentin-1, TNF-α = tumor necrosis factor-α, IL-6 = interleukin-6, TSH = thyroid-stimulating hormone, TC = total cholesterol, LDL = low density lipoprotein, CAD = coronary artery disease, hsCRP = high sensitive c-reactive protein, AI = atherogenic index, BMI = body mass index, BFP = body fat percentage, ELISA = enzyme-linked immune sorbet assay, TG = Total triglyceride, VLDL = very low density lipoprotein.

Introduction

Adipose tissue secretes many adipokines including adiponectin, chemerin, leptin, resistin, retinol binding protein 4, tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) [(1)]. These adipokines play important roles in carbohydrate and lipid metabolism, homeostasis, insulin resistance, diabetes, atherosclerosis, vascular endothelial dysfunction, inflammation, and cardiovascular function [(2,3)]. Omentin, a recently identified fat deposition-specific adipokine codified by two genes (1 and 2), omentin-1 has been shown to be the major circulating isoform in human plasma [(4)].

Omentin-1 or Intelectin-1 (OM-1) is a newly identified protein (32 kDa adipokine) that is highly and selectively expressed in visceral adipose tissue and is expressed to a lesser extent in the heart, lung, and placenta [5].

Dysregulation of (OM-1) secretion is thought to play a role in the pathophysiology of endothelial dysfunction, and cardiovascular disease [6]. (OM-1) may act as an endocrine factor affecting muscles [7].

In clinical studies, circulating (OM-1) concentrations have been shown to be decreased in patients with obesity, impaired glucose regulation, polycystic ovary syndrome, type 1 diabetes, and type 2 diabetes [8-11].

Low circulating levels of (OM-1) have also been associated with endothelial dysfunction and cardiovascular disease [12-17].

Hypothyroidism is caused either by inadequate function of the gland itself (primary hypothyroidism) or by not enough stimulation by thyroid-stimulating hormone (TSH), central hypothyroidism [18,19].

In overt primary hypothyroidism (OHT), TSH levels are high; T4 and T3 levels are low. TSH usually rises after T4 and T3 levels drop.

Subclinical hypothyroidism (SHT) is a milder form of hypothyroidism characterized by an elevated serum TSH level, but with a normal serum free thyroxine level [20]. Hypothyroid patients have increased levels of total cholesterol (TC) and low-density lipoprotein (LDL). Indeed, hypo-thyroidism is a common cause of secondary dyslipidemia [21].

The abnormalities of lipid metabolism associated with (OHT) predispose to the development of atherosclerotic coronary artery disease (CAD) [22]. Moreover, hypothyroidism can adversely affect other CVD risk factors, further contributing to increasing CAD risk. Decreased thyroid function not only increases the number of LDL particles, but also promotes LDL oxidability [23].

In addition, thyroid failure is strongly associated with arterial hypertension (especially diastolic) via sympathetic and adrenal activation, and increased vascular stiffness. Subjects with (OHT) also exhibit impaired endothelial function [24].

We hypothesized that omentin-1 might be implicated in CVD in patients with (SHT and OHT) due to a possible association with inflammation endothelial function. To test the hypothesis, serum (OM-1) levels in women have (SHT and OHT) patients are measured, and compared them with healthy women subject as a control group and evaluated possible correlations with other cardiovascular risk factors such as high sensitive c-reactive protein (hsCRP), lipid profile and atherogenic index (AI).

Methods

Ninety women (age 40-60) years were enrolled in this study, blood was collected from women patients attended the Baghdad Teaching Hospital, Al-Kindy Hospital and Al-Imamain Al-Kadhemain Medical City from March 2011 to September 2012. The women were divided into three groups as follows: group I consist of 30 healthy women as a control group, group II consist of 30 OHT patients and group III consist of 30 SHT patients.

All women patients were diagnosed by physicians and other complications were excluded such as cardiovascular disease, diabetes mellitus, renal failure and hypertension. The range of body mass index (BMI) for the patients and control was 32-38 Kg/m^2 and the range of body fat percentage (BFP) was (30-39), Patients and control groups were determined the following parameters:

- TSH, T3, T4, OM-1 and high sensitive c-reactive protein (hsCRP) levels were measured by enzyme-linked immune sorbet assay (ELISA) method [25,26].
- Total cholesterol (TC) was determined using enzyme-catalyzed colorimetric method [27].
- Total triglyceride (TG) was determined using enzyme-catalyzed colorimetric method [28].
- Serum HDL was measured using Burstein separation method using HDL-C kit [29].
- By using the Friedwald equation, low density lipoprotein (LDL) = TC-[TG/5 + HDL], very low

density lipoprotein (VLDL) = TG/5, atherogenic index of plasma (AIP)= Log(TG/HDL) [30].

Statistical Analysis

Data are presented as mean ±SD using SPSS program. The differences between two groups were analyzed by t-test. *P* value less than 0.05 considered significant. Pearson's correlation coefficient was used to examine between (OM-1) and other parameters in patients groups.

Results

Table 1 shows the level of OM-1, thyroid hormones and lipid profile of the studied groups. The serum level of T3, T4, HDL and OM-1 were significantly lower in the patient than in the control group ($P < 0.05$). The serum level of T3, T4, HDL and OM-1 levels were significantly lower in OHT patients than in the SHT patients ($P < 0.05$).

The serum levels of TSH, hsCRP, TC, HDL, LDL, VLDL, and AI were significantly higher in SHT and OHT patients than in the control group ($P < 0.05$) and TG level was insignificantly higher than the control group.

The serum levels of TSH, hsCRP, TC, HDL, LDL, VLDL, AIP levels were significantly higher in OHT patients than in the SHT patients ($P < 0.05$) and TG level was insignificantly higher than the SHT patients.

Table 1. Omentin-1, thyroid functions, inflammatory markers and lipid profile in the control group and patients with subclinical and overt hypothyroidism patients.

Parameter		Groups			*P* Value		
		I	II	III	I-III	I-II	III-II
Thyroid functions	T3 (ng/mL)	1.2±0.5	0.7±0.2	0.3±0.1	s	s	s
	T4 (ng/dL)	1.7±0.7	0.9±0.34	0.5±0.12	s	s	s
	TSH (mU/L)	1.92±0.8	7.86±3.1	19.34±4.3	s	s	s
OM-1 (ng/mL)		12.3±2.2	9.7±1.4	7.8±1.1	s	s	s
hsCRP (g/L)		2.25±0.42	4.23±1.2	7.81±2.7	s	s	s
Lipid profile	TG (mg/dL)	104.5±28.3	117.3±21.2	129.6±32.8	s	ns	ns
	TC (mg/dL)	166.0±27.3	195.5±36.2	232.8±57.2	ns	s	s
	HDL (mg/dL)	44.1±9.2	36.1±15.8	30.3±12.3	s	s	s
	LDL (mg/dL)	101.0±15.9	119.7±39.6	128.6±45.7	s	s	s
	VLDL (mg/dL)	25.2±6.5	30.3±9.4	37.1±11.4	s	s	s
	AI (mg/dL)	0.34±0.1	0.55±0.13	0.8±0.14	s	s	s

OM-1 = omentin-1, TSH = thyroid-stimulating hormone, TC = total cholesterol, LDL = low density lipoprotein, hsCRP = high sensitive C-reactive protein, AI = atherogenic index, TG = Total triglyceride, VLDL = very low density lipoprotein, HDL = high-density lipoprotein, s = significant (P < 0.05), ns = not significant.

A significant negative correlation was noticed between OM-1 and TSH, hsCRP, TC and AIP in patients with SHT and OHT. A significant positive correlation was seen between OM-1 and HDL in patients with SHT and OHT. No significant positive correlation was observed between OM-1 and T3 and T4 in patients with SHT and OHT. No significant negative correlation between OM-1 TG and LDL in patients with SHT and OHT (Table 2).

Discussion

To our knowledge, the current study, is the first study exploring the serum OM-1 levels in SHT and OHT patients, we sought to determine the relationship between circulating OM-1 levels and T3, T4, TSH, lipid profile and some inflammation in patients groups. AI and HDL cholesterol was found to be a significant correlate of plasma OM-1 concentrations in the entire study cohorts; these data demonstrate that omentin-1 is associated with HDL cholesterol. So it seems that

OM-1 has a role in the pathogenesis of SHT and OHT and their relation to CVD. This finding is in agreement with a recent study by Vu et al (2014) which shows that OM-1 concentration play a role in the developing of CVD [31] and the correlation between HDL cholesterol and omentin-1 has been previously described in the settings of CVD [32].

Table 2. Correlation between Omentin-1 and other parameters for the patient groups

Parameter		Subclinical hyperthyroidism		Overt hyperthyroidism	
		r value	*P* value	*r* value	*P* value
Thyroid function	T3 (ng/mL)	0.01	ns	0.27	ns
	T4 (ng/dL)	0.12	ns	0.33	ns
	TSH (mU/L)	-0.41	s	-0.54	s
hsCRP(g/L)		-0.46	s	-0.63	s
Lipid profile	TG (mg/dL)	-0.21	ns	-0.32	ns
	TC (mg/dL)	-0.53	s	-0.75	s
	HDL (mg/dL)	0.55	s	0.68	s
	LDL (mg/dL)	-0.04	ns	-0.05	ns
	VLDL (mg/dL)	-0.03	ns	-0.11	ns
	AI (mg/dL)	-0.59	s	-0.66	s

OM-1 = omentin-1, TSH = thyroid-stimulating hormone, TC = total cholesterol, LDL = low density lipoprotein, hsCRP = high sensitive C-reactive protein, AI = atherogenic index, TG = Total triglyceride, VLDL = very low-density lipoprotein, HDL = high-density lipoprotein, s = significant ($P < 0.05$), ns = not significant.

Dysregulation of omentin-1 may adversely affect insulin signaling and regulation, thereby altering HDL production and then total cholesterol concentration. Although few prospective data exist for omentin-1, some studies suggest that circulating omentin-1 concentrations are associated with atherosclerosis and CAD in different patient populations [31].

Thyroid hormones are also crucial for the regulation of total energy consumption and body composition besides their roles in normal growth, development, and reproduction.

Thyroid dysfunction is associated with weight changes. A significant negative correlation between OM-1 and TSH was found in this study.

Hypothyroidisms is also associated with insulin resistance and altering HDL-c and other lipid profile [33,34].

OM-1 is discovered to have role in regulation of metabolism and body composition, it seem to regulate thermogenesis, immunity, feeding, and neuroendocrine functions.

In this study, negative correlation of OM-1 with hsCRP was found. OM-1 has a central role in subclinical inflammation of adipose tissue [35-37].

In SHT cases, high serum cholesterol concentration was regarded as evidence for "premyxoedema" in the absence of symptoms of hypothyroidism. How many of these patients in fact had hypothyroidism is not known.

While there is no doubt that many hypothyroid patients have abnormal serum lipid concentrations, the increased risk of CAD seen in hypothyroid patients is likely multifactorial in etiology [38].

In conclusion, SHT patients have obesity, altered lipid profile and high AI and hsCRP levels; all of these conditions are correlated with CVD.

Serum OM-1 levels was decreased in hypothyroidism patients and is correlated with previous parameters, so low OM-1 level is a risk factor for CVD in hypothyroid patients.

We recommend monthly follow up by serum lipid profile for middle age women. OM-1 can be used therapeutically for better management of SHT and OHT to prevent development of CVD.

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Conflict of interest

The author declares that they have no competing interests.

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Health-related Quality of Life (HRQOL) among Women with and without Medical Problems during Last Pregnancy and its Association with Postnatal Depressive Symptoms and Adverse Pregnancy Outcome

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Abstract

Background There is an increasing interest in measuring quality of life in clinical settings and in clinical trials. None of the commonly used quality of life (QOL) instrument had been validated for use postnatally.

Objectives To assess the psychometric properties of the 26-item of World Health Organization Quality of Life –BREF WHOQOL-BREF among women following childbirth and to identify women with postnatal depression by using Edinburgh Postnatal Depression Scale EPDS.

Methods Cross sectional study was carried out during the period from the 1st of December 2013 to 31st March 2014 in Baghdad, Iraq. A total number of 558 women were asked within first 48 hours after delivery to complete a questionnaire form which contains three elements (maternal socio-demographic and obstetrical variables, the WHOQOL-BREF which was developed by the WHO and EPDS).

Result The study showed that the prevalence of postnatal depressive symptoms among 558 mothers was 33.5%. Most pregnant women included in study had fair QOL scores on all domains at late pregnancy. Thus, the study concluded that women who experienced medical diseases such as hypertension, diabetes and other diseases had a lower HRQOL at late pregnancy than those who were apparently healthy. Also, woman with newborns complications' (respiratory distress, low birth weight and other complications) had been experienced significant declines in psychological health.

Conclusion The WHOQOL-BREF is well-accepted instrument in this sample and may be used in postnatal clinical settings or for assessing intervention effects in research studies.

Key words Quality of life, postnatal depression, adverse pregnancy outcome.

List of abbreviation: APH = Antepartum hemorrhage, CS = Cesarean section, GDM = Gestational diabetes, NVD = Normal vaginal delivery, PIH = preeclampsia, PNDS = Postnatal depressive symptoms, QOL = Quality of life, DM = diabetes mellitus, WHO = world health organization, WHOQOL –BREF = world health organization Quality of life –BREF.

Introduction

The Constitution of the World Health Organization (WHO) defines health as "A state of complete physical, mental, and social well-being and not merely the absence of disease". It follows that the measurement of health and the effects of health care must include not only an indication of changes in the frequency and severity of diseases but also an estimation of well-being and this can be assessed by measuring the improvement in the quality of life related to health care [1].

Health related quality of life (HRQOL), on the other hand, includes domains (aspects) of life that improve when a treatment option is successful. A clinically significant change in HRQOL is indicated by a decline in a domain that

leads a physician or health care provider to alter a medication or medical treatment. HRQOL domains minimally include physical state, mental health or emotional well-being. These domains represent typical outcomes in medical and social science research [2,3].

Pregnancy is a specific condition that is not a disease or a normal state of woman's health. There are specific organ and hormonal changes that affect bodily functions during pregnancy and often the overall well-being and sometimes mental well-being of pregnant women. It results in changes to a pregnant woman's quality of life [4,5].

In chronic conditions such as hypertension and diabetes, HRQOL is an especially important outcome, given their life long nature and the need for daily self-management [6].

Hypertension has been shown to be associated with negative outcomes in HRQOL, especially in the domain of subjectively perceived general health, although its impact on HRQOL is usually less adverse than that of other chronic diseases [6].

HRQOL has been found to be poorer in diabetic participants than in the general population, especially in the domains of self-perceived physical health, while findings on domains of psychosocial functioning vary between studies [7].

The term depression describes a spectrum of mood disturbance ranging from mild to severe and from transient to persistent. Depressive symptoms are continuously distributed in any population but are judged to be of clinical significance when they interfere with normal activities and persist for at least two weeks, in which case a diagnosis of a depressive illness or disorder may be made. The diagnosis depends on the presence of two cardinal symptoms of persistent and pervasive low moodand loss of interest or pleasure in usual activities [8].

In the 1st trimester, a woman may have increased emotional liability, which may be exacerbated by nausea, breast tenderness and other physical changes typical of early pregnancy. As pregnancy progresses, further bodily changes, alteration in sexual interests and anxieties about the delivery may all contribute to mood change [9].

Late pregnancy may be associated with social withdrawal and increased absorption and preoccupations with preparations for delivery and caring for the baby [9].

The goal of the study is to assess the psychometric properties of the 26-item WHOQOL-BREF among women with and without medical problems in last pregnancy and to identify the association between HRQOL and postnatal depressive symptoms and adverse pregnancy outcomes.

Method

A cross sectional study was carried out during the period from the 1st of December 2013 to 31st March 2014 in two teaching hospitals in Baghdad Al-Karkh (Al-Imamain Al-Kadhimain Medical City and Al-Yarmouk Teaching Hospital). This study was endorsed by the Institute Review Board of the College of Medicine, Al-Nahrain University. A sequential sample was obtained, which include 558 womenadmitted for delivery in both hospitals during the study period. All women were interviewed within first 48 hours after delivery, all of them had term labor (37 weeks or more) delivered by normal vaginal delivery (NVD) or caesarian section (CS).

Women included in this study were either apparently healthy or with medical problems during last pregnancy like gestational diabetes mellitus (GDM), essential hypertension, preeclampsia (PIH) and other diseases. Women with antepartum hemorrhage (APP), multiple pregnancy and previous history of psychological disorder or non-cooperative women were excluded from this study.

Mothers who meet the inclusion criteria were interviewed by the researcher using a structured questionnaire form which is constructed for this study. To fill the questionnaire form, each mother was interviewed after signing a consent form that declared her voluntary agreement to participate in this study. The nature and objectives of the study was explained and

assurance regarding confidentiality was confirmed.

Pilot study was carried out on six women who had delivered at the Al-Imamain Al-Kadhimain Medical City to test the feasibility of the questionnaire, estimate the time needed to fill it and to identify some terms and words used by public and understood by Iraqi mothers.

The questionnaire form consists of three sections:

The first section

It includes questions related to maternal variables: socio-demographic, obstetrical, antenatal care, medical problems at last pregnancy and maternal and newborns' complications.

The second section

The world health organization quality of life WHOQOL-BREF was developed by the WHOQOL Group, in 15 international field centers [10]. It is self-report questionnaire using the same local language of the mother, it contains 26 items, and each item represents one facet. The facets are defined as those aspects of life that are considered to have contributed to a person's quality of life and represent what she think about her life in the last four weeks [10].

Among the 26 items, 24 of them make up the 4 domains of physical health (7 items), psychological health (6 items), social relationships (3 items), and environment (8 items). The other 2 items measure overall quality of life and general health [10] and scored from 1-5 while questions 3, 4 and 26 were reversed according to the guidelines for the world health organization Quality of life –BREF WHOQOL-BREF [11].

The scores of items within each domain are used to calculate domain scores. The score in each domain (subscale) is calculated by adding up the scores of the corresponding items. The overall QOL score is the summation of all four subscale score plus another two global item scores [12]. The QOL score is then used to classify the quality of life as bad, fair or good as presented in table 1 [13].

Table 1. Subscale and overall quality of life scoring criteria

Subscale	Good	Fair	Bad
Physical domain	27-35	17-26	7-16
Psychological domain	23-30	15-22	6-14
Social domain	12-15	8-11	3-7
Environmental domain	30-40	19-29	8-18
Overall	96-130	61-95	26-60

The internal consistency, discriminant validity and correlation matrix of the WHOQOL –BREF of the questionnaire were checked. Internal consistency was determined by calculating Cronbach's Alpha for each of the four domains, with an acceptable value set at > 0.70 [14].

Correlation between the individual items of the WHOQOL-BREF and the four domains was assessed using a 2-tailed Pearson correlation coefficient, we accepted a moderate correlation between ($r \geq 0.45$ to $r < 0.70$) [15,16].

The third section

Edinburgh Postnatal Depression Scale (EPDS) had been used to identify mothers with postnatal depressive symptoms. The mother is asked to check the response that comes closest to how she has been feeling in the previous 7 days [17]. It includes 10 questions; Mothers who score ≥ 12 are likely to be suffering from a depressive illness [17].

Statistical analysis:

Data of the study had been analysed using available statistical computer program of statistical package for social sciences (SPSS-16). Mean scores of each domain of QOL had been calculated. For discriminate validity we used *t*-tests to examine the ability of the WHOQOLBREF to detect differences between groups. The groups were those scoring ≥12 on the EPDS (PND

group) and those scoring < 12 on the same instrument (the non-PND Group). Also Chi-square test had been used for the assessment of the association between two categorical variables and student *t*-test for continuous data had been used to test the significance differences of two means and ANOVA for testing the difference among three means and above. An association or difference had considered statistically significant if the probability value (*P* value) is less or equal to 0.05 [(18)].

Results

The highest proportion of women studied aged between 20-24 years (31.7%), about two thirds of the study sample were from urban area (78.3%). Similar proportions of women and their husbands have finished primary school (53.2% and 49.5% respectively). Majority of women were housewives (93.2%), while 64.9% of their husbands were workers. The family type of 64.9% of women was extended, the rest had nuclear families.

Regarding obstetrical history, about 66% of women studied had parity range of 2-5, history of previous abortion was found in 150 women (26.9%), and the rate of women who were delivered by caesarean section was 76.9%. Women who had antenatal care (4 visits) during last pregnancy constituted 79%. The rate of women had unintended last pregnancy was 60.6%. More than half of Mothers were without medical problems during last pregnancy (52.2%), while those with hypertension, diabetes, and other diseases during pregnancy were 18.5%, 7% and 22.6% respectively.

The homogeneity between items on each of the sub-scales was measuredwith the alpha coefficient for each domain of the WHOQOL-BREF exceeding 0.70 as presented in Table 2. The only exception was in the Environmental and social domains where the alpha coefficient was 0.586 and 0.455 respectively.

Table 2. Domain score distribution statistics

Quality of life domain	Minimum	Maximum	Mean±SD	Cronbach's Alpha
Physical domain	12	29	21.54±3.68	0.808
Psychological domain	13	29	21.88±3.62	0.729
Social Relationships	5	13	8.99±2.00	0.455
Environment	15	32	26.82±3.19	0.586

The domain structure of the WHOQOL-BREF was found to be valid in this sample of mothers. There was moderate to high correlation between individual items and the domain structure to which the items were originally assigned. Except for question 8, 13, and 14 which was equal to 0.25, 0.29, and 0.38 respectively as presented in table 3.

Table 4 shows the ability of the WHOQOL-BREF to clearly discriminate between known groups. Women scoring < 12 on the EPDS scored higher than those scoring above 12 in all domains and these differences were statistically significant. Discriminant validity was strongest in the psychological domain.

The study showed that the prevalence of postnatal depressive symptoms among mothers was 33.5%. A significant association was found between postnatal depressive symptoms and some variables such as occupation of mothers, family type, parity, mode of delivery, health status of mothers and newborns' complicationsas presented in table 5.

Most pregnant women included in study had fair QOL scores on all domains at late pregnancy. The overall QOL show that most of the mothers fairly well, with 17.4 % had good score, 82.4 % had fair score and 0.2 % had poor score. For physical activity domain 11.1 % had poor score, for psychological domain 0.7 % had poor score, for social domain 22.4 % had poor score and environmental domains 1.6 % had poor score as presented in table 6.

Table 3. Correlation matrix for the four domains of the WHOQOL-BREF*

WHOQOL-BREF items	Physical health	Psychological health	Social health	Environmental health
Physical health Pain(3)	0.74	0.35	0.27	0.18
Dependence of medical aids (4)	0.56	0.20	0.17	0.03
Energy (10)	0.83	0.42	0.30	0.20
Mobility (15)	0.59	0.19	0.20	0.11
Sleep and rest (16)	0.48	0.27	0.25	0.11
Activity of daily living (17)	0.85	0.41	0.32	0.18
Work capacity (18)	0.80	0.37	0.26	0.16
Psychological health Enjoyment of life (5)	0.28	0.82	0.42	0.30
Personal belief (6)	0.26	0.83	0.44	0.31
Concentration (7)	0.21	0.52	0.22	0.12
Bodily image (11)	0.18	0.49	0.13	0.16
Self- esteem (19)	0.74	0.47	0.29	0.30
Negative feeling (26)	0.26	0.77	0.43	0.20
Social relationships - personal relationship (20)	0.30	0.46	0.75	0.18
Sexual activity (21)	0.33	0.31	0.70	0.15
Social support (22)	0.11	0.23	0.62	0.23
Environmental health Security (8)	0.15	0.24	0.11	0.25
Physical environment (9)	0.12	0.23	0.20	0.73
Financial support (12)	0.16	0.20	0.15	0.62
Accessibility of information (13)	0.11	0.13	0.13	0.29
Leisure activity (14)	0.12	0.13	0.19	0.38
Home environment (23)	0.11	0.21	0.19	0.72
Health care accessibility (24)	0.10	0.14	0.13	0.45
Transport (25)	0.14	0.20	0.19	0.49

*Correlation of ≥0.45 was considered satisfactory

Table 4. Mean scores of four domains of WHOQOL-BREF of women with and without PNDS

Domain	Non PNDS	PNDS	*P* value
Physical domain	22.21±3.614	20.19±3.485	<0.01
Psychological domain	23.69±2.847	18.23±2.400	<0.01
Social Relationships	9.62±2.009	7.89±1.814	<0.01
Environment	27.57±2.865	25.32±3.301	<0.01

Women who experienced medical diseases such as hypertension, DM and other diseases had a lower health-related quality of life at late pregnancy compared to those who were apparently healthy at physical, psychological and social health (Table 7). In addition, woman with newborns complications' (respiratory distress, low birth weight and other complications) had been experienced significant declines in psychological health as presented in table 8.

Table 5. Postnatal depressive symptoms in relation to socio-demographic variables and pregnancy outcomes

Variables		Non –PNDS N = 371 (%)	PNDS N = 187 (%)	Total N = 558 (%)	Chi-square	*P* value
Age (year)	≤ 19	50 (72.5)	19 (27.5)	69 (100)	5.448	>0.05
	20-24	122 (68.9)	55 (31.1)	177 (100)		
	25-29	89 (69)	40 (31)	129 (100)		
	30-34	51 (58.6)	36 (41.4)	87 (100)		
	>35	59 (61.5)	37 (38.5)	96 (100)		
Residency	Urban	292 (66.8)	145 (33.2)	437 (100)	0.100	>0.05
	Rural	79 (65.3)	42 (34.7)	121 (100)		
Occupation of mother	Housewife	338 (65)	182 (35)	520 (100)	9.737	<0.05
	Employed	27 (93.1)	2 (6.2)	29 (100)		
	Others	6 (66.7)	3 (33.3)	9 (100)		
Education of mother	Illiterate	55 (71.4)	22 (28.6)	77 (100)	7.340	>0.05
	Primary or less	186 (62.6)	111 (37.4)	297 (100)		
	Secondary school	86 (66.7)	43 (33.3)	129 (100)		
	College or higher	44 (80)	11 (20)	55 (100)		
Type of family	Nuclear	111 (56.6)	85 (43.4)	196 (100)	13.168	<0.05
	Extended	260 (71.8)	102 (28.2)	362 (100)		
newborns' complications	Yes	95 (58.6)	97 (41.4)	162 (100)	6.306	<0.05
	No	276 (69.7)	120 (30.3)	396 (100)		
Types of newborns' complications	None	276 (69.7)	120 (30.3)	396 (100)	23.139	<0.05
	Low birth weight	37 (62.7)	22 (37.3)	59 (100)		
	Respiratory distress	38 (52.8)	34 (47.2)	72 (100)		
	Neonatal jaundice	16 (94.1)	1 (5.9)	17 (100)		
	Others	4 (28.6)	10 (71.40)	14 (100)		

Table 6. Quality of life (QOL) and subscale QOL scores of mothers

Quality of life domain	Quality of life level		
	Good	Fair	Poor
Physical domain	35 (6.3%)	461 (82.6%)	62 (11.1%)
Psychological domain	251 (45%)	303 (54.3%)	4 (0.7%)
Social Relationships	89 (15.9%)	344 (61.6%)	125 (22.4%)
Environment	123 (22%)	426 (76.3%)	9 (1.6%)
Overall Quality	97 (17.4%)	460 (82.4%)	1 (0.2%)

Table 7. Score domains of WHOQOL-BREF according to health status of mothers

Health status of mothers		Healthy	HT	DM	others	ANOVA test	*P* value
Domain	Physical	22.53±3.432	20.65±3.353	20.08±4.061	20.44±3.738	F=16.026	0.000
	Psychological	22.48±3.598	21.22±3.608	21.26±3.891	21.21±3.890	F =5.735	0.001
	Social	9.27±1.982	8.72±1.864	9.21±2.262	8.50±1.963	F =5.306	0.001
	Environmental	26.88±3.191	26.83±3.001	26.95±3.260	26.63±3.329	F =0.193	0.901

HT = hypertension, DM = DM

Table 8. Score domains of WHOQOL-BREF according to newborns' complications

Newborns' complications		None	Low birth weight	Respiratory Distress	Jaundice	Others	ANOVA test	*P* value
Domain	Physical	21.76±3.639	21.27±3.800	20.68±3.845	20.76±3.327	21.86±3.325	F=1.632	0.165
	Psychological	22.09±3.569	21.93±3.800	20.992±3.519	22.35±3.499	19.64±4.011	F=2.875	0.02
	Social	9.05±2.013	8.56±2.011	8.89±1.903	9.35±2.499	9.07±1.269	F=0.974	0.421
	Environmental	26.91±3.132	26.66±3.911	26.08±3.001	27.88±2.497	27.36±2.706	F=1.658	0.158

The mean score of QOL in all domains was inversely related to increasing age of the mothers, there was statistically significant difference in both physical and environmental domains. The mean score of QOL in all domains was inversely related to increasing parity of the mothers and increasing of depression score, there was statistically significant difference in all domains as presented in table 9.

Table 8. Age, parity, and depressive scores of women among the domains of WHOQOL- BREF

Variables	Physical domain			Psychological domain			Social Relationships			Environment		
	Good	Fair	poor	Good	Fair	Poor	Good	Fair	Poor	Good	Fair	Poor
Age (years) Mean±SD	25.20 6.512	26.61 6.867	29.29 6.867	26.56 6.868	26.99 6.530	29.75 4.787	26.46 6.784	26.56 6.514	27.78 6.983	25.65 6.757	27.05 6.603	31.56 6.187
P value	<0.05			>0.05			>0.05			<0.05		
Parity Mean±SD	2.26 1.578	2.83 1.745	3.45 1.762	2.63 1.711	3.05 1.773	3.25 1.258	2.52 1.989	2.82 1.623	3.23 1.863	2.18 1.349	3.03 1.808	4.33 1.118
P value	<0.05			<0.05			<0.05			<0.05		
Depression score Mean±SD	5.80 3.962	8.83 4.499	11.18 5.309	5.14 2.830	11.88 3.442	18.50 3.416	4.47 3.170	8.94 4.299	11.95 4.105	6.11 3.955	9.61 4.535	13.44 5.270
P value	<0.05			<0.05			<0.05			<0.05		

Discussion

The studies in other countries such as Iran, Kuwait and Malawi had noted problems with internal consistency of the social relationships domain [16,19,20] and also the WHOQOL-BREF field trial reported a Cronbach's alpha less than 0.7 in this domain [10]. This can be attributed to the small number of questions (3 items) in this domain. In addition, this domain does not appear very homogenous at least in the Iraqi culture, since it inquires about sexual life and social supports, which are relatively different concepts in Iraqi culture.

In this study, the alpha coefficient for environmental domain was less than 0.70, this can be attributed mainly to dissatisfaction with security and health care accessibility. This could be attributed to the fact that Iraqi population was exposed to wide spread violence in last years.

The domain structure of the WHOQOL-BREF was found to be valid in this sample of mothers. There was moderate to high correlation between individual items and the domain structure to which the items were originally assigned. Except for question 8 (security), 13 (accessibility of information) and 14 (Leisure activity) which was equal to 0.25, 0.29, and 0.38 respectively.

A study done in Malawi found that all 27 items were correlated to the domain to which they are assigned, with all correlations greater than, or equal to 0.60 [20]. This value was higher than study done in Iran, which was equal to 0.40 [16].

In the WHO 23-country report [10], it was noted that in 7 of 24 centers, the items on pain and the need for medical treatment were generally problematic in item-total correlations in the physical domain. In addition, poor item-total correlation (<0.30) was noted for negative feelings in one center.

In terms of discriminant validity the WHOQOL-BREF performed particularly well. On average, in each of the domains, women in the non-PND group had higher scores than those with EPDS scores over 12. And this is similar to study done in Australia, Kuwait and also the WHOQOL-BREF field trial [10,19,21].

It was also interesting to note the sensitivity of the instrument in terms of the strength of difference between the PND group and the non-PND group for each of the domains. The WHOQOL Group suggests that discriminant validity is best demonstrated in the physical domain [10]; however, in this study, the psychological domain showed the greatest difference between groups and this similar to that found in Australia [21].

In this study, the indicator used to determine postnatal depressive symptoms was EPDS with a cut-off score of ≥ 12 for depressive symptoms. In the prevalence of postnatal depressive symptoms among 558 mothers was 33.5%. This is approximately similar to study done in Bahrain, which found that prevalence of PND was 37.1% [22].

This result was in disagreement with that reported in Erbil, Lebanon and Karachi with overall prevalence of PPD; 28.4%, 21% and 52.21% respectively [23,24,25].

In this study, women who experienced hypertension had a lower health-related quality of life at late pregnancy than those who were apparently healthy. This finding is similar to other study done in Canada, which showed women experience lower HRQOL during pregnancy, particularly in the physical domain [26].

Another study was done in San Francisco also showed women with PIH more often reported a significant decline in HRQOL and an increase in depressive symptoms from pre-pregnancy to postpartum compared with unaffected women [27].

This decline in health related quality of life was mainly due to most pregnant women with gestational hypertension ended their pregnancy with cesarean section.

In this study, women who experienced diabetes had a lower health-related quality of life at late pregnancy than those who were apparently healthy. This finding is similar to that of a study done in Italy, which showed that pregnancy is associated with a perception of poor general health in women with both type 1 DM and GDM. After delivery, significantly worse depressive symptoms were documented in both groups, while a generally worse physical and psychological well-being was only identified in women with type 1 DM [28].

On the other hand, this result disagreed with that from San Francisco, which showed that women with GDM were not experience significant declines in health status compared to unaffected women [27].

This could be explained by a sense of control over the disease is an important factor influencing the mood of patients, their functioning and their health. The adaptation to the disease affects the way it is perceived by the patient. Women suffering from gestational diabetes, as the disease affecting their lives, have problems to come to introduce the changes needed to treat, such as adjustments to the new dietary recommendations, measurement of blood glucose, frequent medical checks and treatment with insulin constitute most difficulties to these patients [29].

In this study, a woman with newborn complications (respiratory distress, low birth weight and other complications) experience significant decline in health status of psychological domain compared to women

without these complications. Other domains did not show significant declines in health status of mothers with newborns complications' compared to women without these complications.

This finding is similar to study done in Taiwan that showed the pregnant women who had a very low score on mental health had a higher risk of giving birth prematurely than did women who had higher scores and mothers with poor mental health can predict low birth weight [30].

In conclusion, the WHOQOL-BREF is well-accepted instrument in this sample and may be used in postnatal clinical settings or for assessing intervention effects in research studies.

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Author contributions

Dr. Najlaa did the data collection, analysis and writing and Dr. Maysaloun made the conception and design, interpretation, and revision of the manuscript.

Conflict of interest

None.

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Studying the Frequency of Methicillin-Resistant *Staphylococcus aureus* Through the Molecular Detection of *mec*A

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Abstract

Background Methicillin-resistant *Staphylococcus aureus* is a major cause of serious nosocomial infections and it is very important to have a reliable test to detect these bacteria. *mec*A encodes the penicillin binding protein 2a, which is associated with staphylococcal methicillin resistance.

Objective The study was to determine the frequency of methicillin-resistant *Staphylococcus aureus* in different specimens from Iraqi patients and to genetically characterize and type the samples of methicillin-resistant *Staphylococcus aureus* through the detection of *mec* A gene.

Methods Sixty clinical isolates of *Staphylococcus aureus* were submitted to DNA extraction. Genomic DNA was submitted to conventional polymerase chain reaction assays, employing MR1-MR2 primers (primer set). The results were compared to the cefoxitin disks agar diffusion method.

Results Fifty seven of the sixty isolates showed positive results for *mec*A amplification while three isolates (5%) showed negative results for *mec*A gene.

Conclusion Good correlation between the *mec*A gene detection by PCR and the cefoxitin disk diffusion methods was obtained.

Key words *mec*A gene, *Staphylococcus aureus*, methicillin resistance *Staphylococcus aureus*

List of abbreviation: MRSA = Methicillin-resistant *Staphylococcus aureus*, PBP2a = penicillin-binding protein2a, OSS = oxacillin salt screening, PCR = polymerase chain reaction

Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) is a major cause of the increasingly prevalent, difficult and expensive-to-treat nosocomial infections worldwide. Methicillin-resistance in staphylococci constitutes resistance to all of the β-lactam antibiotics and their derivatives. The major mechanism of resistance is the acquisition of the *mec*A gene that encodes for additional penicillin-binding protein2a (PBP2a) [(1)]. The phenotypic methods such as cefoxitin disk agar diffusion and oxacillin salt screening test (OSS) are widely used in routine microbiological laboratory for the detection of MRSA [(2,3)]. The problem with phenotypic methods is that they can be influenced by culture condition such as temperature, medium pH and NaCl content in the medium [(4)]. Several PCR-based methods have been developed to detect the *mec*A gene [(1,5)]; however, one pair of PCR primers are most commonly used [(1,6)]. The purpose of the present work was to determine the frequency of MRSA in different specimens from Iraqi patients and to genetically characterize and type the isolated MRSA through the detection of *mec*A gene.

Methods

Bacterial isolates

One hundred and five samples were collected from different body sites and lesions (UTI, wounds, and ear swabs...etc.) of in-and-out patients from both sexes who attended Al-Imamain Al-Kadhimain Medical City, Baghdad during the period from November-2012 to March-2013. The isolates were identified using standard microbiological procedures [(7)] in Medical Microbiology Laboratories of College of Medicine, Al-Nahrain University. API Staph System, produced by bio-Merieux was also used for the confirmation of the primary identification.

Cefoxitin sensitivity test

According to the method described by Murakami [(1)], olonies from an overnight nutrient agar plate culture were transferred to a tube containing 3 ml of 0.85% normal saline**.** The turbidity was adjusted to 0.5 McFarland equal to 1.5×10^8 CFU/ml.

A sterile cotton swab was dipped into the bacterial suspension; excess fluid was removed by pressing the swab against the tube wall. The bacterial suspension was inoculated into Muller Hinton agar plates and the plates were left to dry for 10 minutes. Each individual petri dish was divided into two sections so as to test two different isolates simultaneously. The antimicrobial discs of cefoxitin (30 µg) were placed on the surface of the medium using sterilized forceps and the plates were incubated at 37 ^{o}C for 24 hour.

When the incubation was complete, the zones of inhibition around the disks were measured and compared with the break points of Clinical Laboratory Standards Institute (CLSI) [(2)] and decided as susceptible (S) and resistant (R).

DNA extraction

DNA was extracted according to Vogelstein [(8)] adopted by the manufacturer of DNA extraction kit **(Geneaid)®.**

Step 1: Cell Harvesting/Pre-lysis

Bacterial cells (1×10^9) were transferred to a 1.5 ml microcentrifuge tube and they were centrifuged for 1 minute at 14-16,000 xg and the supernatant was than discarded. Two hundred µl of lysozyme buffer were added to the tube and the cell pellet was re-suspended by vortexing or pipetting, after that, the lysate was incubated at room temperature for 10 minutes. During incubation, the tube was inverted every 2-3 minutes. Lysis Step of the Cultured Cell Protocol was then preceded.

Step 2: Cell Lysis

Two hundred µl **of GB Buffer** were added to the sample and vortexed for 5 seconds. It was incubated at 70 ^{o}C for 10 minutes or until the sample lysate is clear. During incubation, the tube was inverted every 3 minutes. At this time, the required **Elution Buffer** (200 µl per sample) was incubated at 70 ^{o}C (for Step 5 DNA Elution).

RNA Degradation

After incubation in water bath at 70ºC, 5 µl of RNaseA (10 mg/ml) was added to the sample lysate and mixed by vortex. Then, it was incubated at room temperature for 5 minutes.

Step 3: DNA Binding

Two hundred µl of absolute ethanol was added to the sample lysate and vortexed immediately for 10 seconds. Precipitate was broken by pipetting. GD Column was placed in a 2 ml collection tube; all of the mixture (including any precipitate) was transferred to the GD column. The mixture was centrifuged at 14-16,000 xg for 2 minutes and 2 ml collection tube containing the flow-through was discarded and the GD column was placed in a new 2 ml collection tube.

Step 4: DNA Washing

Four hundred µl of W1 Buffer were added to the GD column. The mixture was centrifuged at 14-16,000 xg for 30 seconds. The flow-through was discarded and the GD column was placed again in the 2 ml collection tube, six hundred µl of Wash Buffer was added to the GD column. The mixture was centrifuged at 14-16,000 x g for 30 seconds. The flow-through was discarded and the GD column was placed again in the 2 ml

collection tube, to dry the column matrix, it was centrifuged for 3 minutes at 14-16,000 x g.

Step 5: DNA Elution

The dried GD column was transferred to a clean 1.5 ml microcentrifuge tube. One hundred (100) µl of preheated elution buffer or TE were added to the center of the column matrix. Let stand for 3-5 minutes or until the elution buffer or TE is absorbed by the matrix. The final step was the centrifugation at 14-16,000 xg for 30 seconds to elute the purified DNA.

Polymerase chain reaction PCR

The sequence of oligonucleotide primers (MR1 and MR2) that were used in conventional PCR to detect the presence of *mec*A was according to [9] and synthesized in Bioneer® (South Korea).

MR1 F GTG GAA TTG GCC AATACA GG
MR2 R TGA GTT CTG CAG TAC CGG AT

The DNA template of *S. aureus* was prepared and the primers (*mec*A gene) were diluted by adding nuclease free water according to the manufacturer instructions. The master mix contents were thawed at room temperature before use. One µl of template DNA was transferred to master mix tubes which contained 5µl of master mix ; 1µl of the diluted primers was added to the tubes. The volume was completed to 20 µl with Deionized Nuclease – Free water (12 µl for conventional PCR) as shown in table 1.

Table 1. Composition of PCR reaction mixture used for amplification of *mecA* gene (Convetional PCR)

Reagents	Volume (µl)	Concentration (pmol) *mecA* gene
Forward Primer	1	0.988
Reverse primer	1	1.042
DNA template	1	
PCR mastermix	5	2x
(DNAse free) water	12	
Total volume	20	

Tubes were then spun down with a mini centrifuge to ensure adequate mixing of the reaction components. The tubes were placed on the PCR machine and the PCR program, with the right cycling conditions pre-installed, was started. Cleaver Scientific Thermal Cycler TC32/80 was used for all PCR amplification reactions. PCR mixture without DNA template (non-templete negative control) was used as negative controls (Table 2).

Table 2. The PCR thermocycler program for *mecA*

Steps	Temp.	Time	Cycles
Initial denaturation	94 °C	5 min	
Denaturation	94 °C	60 sec	30
Annealing	60 °C	60 sec	
Elongation	72 °C	70 sec	
Final extension	72 °C	10 min	
Hold	4 °C		

Electrophoresis

DNA samples were electrophoresed by horizontal agarose gel electrophoresis according to Sambrook and Russell [10] Agarose at concentrations of 2% was prepared, the agarose was left to cool at 60 °C before adding ethidium bromide in a concentration of 0.5 µg/ml and poured into the taped plate. A comb was placed near one edge of the gel. The gel was left to harden until it became opaque; gently the comb and tape were removed. TBE buffer (0.5X) was prepared, poured into the gel tank and the slab was placed horizontally in electrophoresis tank. About 5 µl of prepared loading buffer, was applied to each 10 µl of DNA sample. The wells were filled with the mixture by a micropipette and adding 5 µl of 100bp DNA ladder to one well. The power supply was set at (5 V/cm (70) for 1 hour) for genomic DNA and PCR products electrophoresis.

Results

Cefoxitine sensitivity testing

By the disc-diffusion method, Staphylococcal isolates were tested for their sensitivity to

cefoxitine (30 mg). A zone of inhibition with a diameter of ≤ 21 mm was considered as an indication for resistance to methicillin (Fig. 1).

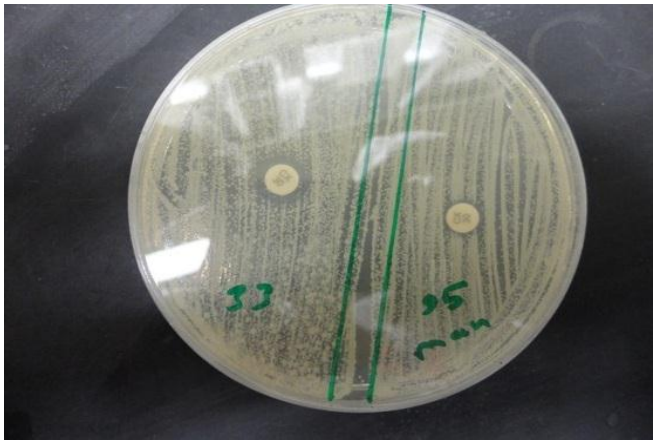

Fig. 1. Antibiotic sensitivity profile of the isolates enrolled in the current study. A zone of inhibition with a diameter of ≤ 21 mm was considered resistant to methicillin

Out of the one hundred and five samples enrolled in this study there were only sixty samples (57.1%) showed resistance to cefoxitin (30 mg) and were considered as methicillin resistant *Staphylococcus aureus*.

DNA extraction

The final concentration of extracted DNA ranged from 4.9 to 167.8 ng/µl and purity ranged from 1.17 to 1.9.

Polymerase Chain Reaction (PCR)

Conventional PCR screening for *mecA* gene

The results of the present study indicated that *mec*A was successfully amplified in fifty seven out of the sixty MRSA isolates, with a product size of 1339 bp, (95 %) of the isolates while only three isolates (5%) lacked this gene and gave negative amplification results (Fig. 2).

Discussion

The frequency of MRSA in many countries is increasing in hospitals in some areas, more than half of all *S. aureus* disease isolates are MRSA. MRSA strains are becoming increasingly multi-resistant, and have developed resistance to vancomycin, used successfully to treat MRSA for more than 30 years. Nosocomial methicillin-resistant *Staphylococcus aureus* infections represent a major challenge to hospital microbiologists because of the emergence and spread of clones with decreased susceptibility to many antibiotic classes. Since the mid to late 1990s, hospital MRSA isolates have increased in prevalence in Europe, the USA and elsewhere (European Antimicrobial Resistance Surveillance System (EARSS) annual report 2001) [(11)].

In one European study of 25 university hospitals, one-quarter of 3051 *S.aureus* isolates collected were MRSA, with a geographical bias towards higher rates in southern countries such as Italy (50.5%) and Portugal (54%), and lower rates in northern European countries, including Netherlands (2%), Austria (9%) and Switzerland (2%) [(11)]. Epidemiological data on MRSA in Africa are scarce. The prevalence of MRSA was determined in eight African countries between 1996 and 1997 and was relatively high in Nigeria, Kenya, and Cameroon (21 to 30) and below 10% in Tunisia and Algeria [(12)]. In Algeria, the rate of MRSA increased to 14% in 2001. MRSA infections are associated with increased morbidity; mortality and length of hospital stay, and represent a major financial burden on healthcare services [(13)].

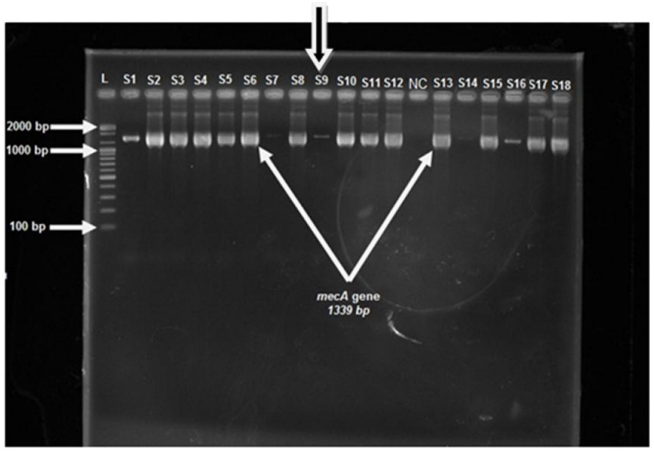

Fig. 2. Gel electrophoresis of conventional PCR products of *mecA*gene (1339bp); negative control; MW,2000 bp ladder; (2% agarose, 5v/cm (70)1hr), the (N.C) between S12 and S13.

The rapid development of resistance is due to mutational events and/or gene transfer and

acquisition of resistance determinants, allowing strains to survive antibiotic treatment. Methicillin-resistant staphylococci depend on efficient penicillin binding protein (PBP2′) production and are modulated by chromosomal factors. Depending on the genetic background of the strain that acquired *mecA*, resistance levels range from phenotypically susceptible to highly resistant. A common characteristic of most methicillin-resistant staphylococci is the heterogenous expression of resistance, which is due to the segregation of a more highly resistant subpopulation upon challenge with methicillin. Maximal expression of resistance by PBP2′ requires the efficient and correct synthesis of the peptidoglycan precursor. Genes involved in cell wall precursor formation and turnover, regulation, transport, and signal transduction may determine the level of resistance that is expressed [(14)].

Detection of the *mecA* gene or its product, penicillin binding protein (PBP2a), by PCR is considered the gold standard for MRSA detection [(15)]. In this study, however, three PCR negative isolates out of the total sixty isolates enrolled in the present study were recorded and this might be explained by some other mechanism rather than the absence of the *mec*A gene. These mechanisms are: 1) *mec*-encoded resistance, 2) overproduction of penicillinase and 3) modifications of normal penicillin-binding proteins [(16)].

Oxacillin may fail to detect them while cefoxitin is strong inducer for production of PBP2a, and do not appear to be affected by hyper-production of penicillinase which may show methicillin resistant [(17)]. Further, cefoxitin has higher affinity for staphylococcal PBP4 than that for PBP2 and overproduction may also contribute in methicillin resistant [(18)]. The present study emphasized the use of a cefoxitin disc diffusion (DD) test for the detection of meticillin resistance in staphylococci. A total of 60 clinical isolates of *Staphylococcus aureus* showed resistant cefoxitin (30 mg disc) as an indication for methicillin resistance. The sensitivity and specificity of the cefoxitin DD test were (95%). The accuracy of the cefoxitin DD test was better than that of the oxacillin DD test for the detection of MR staphylococci. It also does not require special testing conditions such as a lower incubation temperature (35 ^{o}C) and NaCl supplementation in the testing media, as required by the oxacillin DD test [(19)]. Cefoxitin is considered to be a better predictor than oxacillin for the detection of heteroresistance because it is a stronger inducer than oxacillin of penicillin-binding protein 2a (PBP2a) [(18)].

In the current study, sixty samples of 105 showed resistance to cefoxitin (30 mg) (57.1%) and can be considered as methicillin resistant *Staphylococcus aureus*. The widespread emergence of MRSA, especially in various types of nosocomial infections, is a serious clinical problem worldwide. The incidence of methicillin resistance among nosocomial isolates of *S. aureus* is higher than 70% in some Asian countries such as Taiwan, China, and Korea (20).

It is concluded from this study that there is good correlation between the *mec*A gene detection by PCR and the cefoxitin disk diffusion methods.

After this research it is recommended that a large scale multi-center studies are being done both in human patients and normal healthy population to determine more precisely MRSA prevalence depending on mec *A* gene and depending on this study purpose of determination the frequency of methicillin-resistant *Staphylococcus aureus* (MRSA) in different specimens from Iraqi patients and to genetically Characterize and type the samples of methicillin-resistant *Staphylococcus aureus* (MRSA) through the detection of *mec* A gene.

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Author Contribution

Rafeef conducted the sampling, isolation, and molecular work. Ahmed and Amir guided and finished writing and editing the study.

Conflict of Interest

The authors declare no conflict of interest.

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Coccidioidal Meningitis: Case Report

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Abstract

Coccidioidomycosis is caused by the dimorphic fungi of the genus *Coccidioides* (*C. immitis* and *C. posadasii*), which are endemic in desert regions of the southwestern United States, and Central and South America. Meningitis is the most lethal complication of coccidioidomycosis and thus is crucial to recognize. A 64 years old diabetic patient suffering from meningitis was studied. Cerebrospinal fluid (CSF) sample was collected by lumber puncture technique under aseptic conditions from female patients who admitted to Baghdad City hospital, conventional methods included cell count and differentiation, biochemical analysis, staining and culturing of CSF sample was applied. CSF smear with lactophenol cotton blue, revealed a typical picture of Coccidioidis spp. This is the first case reported in Iraq. In conclusion, fungal meningitis should be included as one of the most causes in chronic meningitis in Iraq.

List of abbreviations: DM= diabetes mellitus, CSF = cerebrospinal fluid, CT scan = computerized tomography scan, WBC = white blood cells, PMN = poly morphonuclear cells, TB = tuberculosis, SPP. = species, IV = intravenous.

Introductions

Coccidioidomycosis is a disease result from the infections with dimorphic fungi called *coccidioides* which have ability to disseminated from cutaneous to other organs and the clinical entity was recognized by Wernicke and Posadas in Argentina in 1882 [(1)] soon after Rixford and Gilcrist was reported this disease in Calfornia [(2)]. Coccidioidalmeningitis first recognized by Ophuls in 1905 [(3)] Evans [(4)] provided further description in 1909. Ryfkogel discovered the first patient complained from coccidiodal meningitis with hydrocephalus [(5)] Veterans Affairs Armed Forces initiate studies from 1955-1958 that includes the definitive description of coccidioidal meningitis from the pretherapy era [(6)].

This disease is mainly found in the Western Hemisphere, mainly in northwestern Mexico and southwestern United States. The incidence of the disease in the endemic area not known exactly but it relatively stable in the middle decades of the 20th century the reasons for that explained by population growth and migration and by increased the number of immunocompromised hosts, the absolute and relative frequency of primary disease and consequent dissemination have multiplied [(7)].

The persons with Coccidioidal infections are mainly asymptomatic. Primary infections are almost universally to pulmonary and they manifest Influenza-like, pneumonic, and pleural presentations are the most common. If one takes into account the large number of asymptomatic infections, the rate of dissemination is low. In high-risk, symptomatic patients, the dissemination rate can be >15% [(8)].

Coccidioidesimmitis the first species was identified from the genus *Coccidioides*but in recent years Taylor *et al*. recognized a second species, *Coccidioidesposadasii*, which is a more common in Texas, Central and in South America, will the *C. immitis* is more common in California.

Both species are found in Arizona. The clinical manifestations between two species have no distinctions [9].

The clinical presentation of coccidioidal meningitis is similar to the other form of meningitis, which included headache. Alteration in mental acuity, with or without fever, vomiting, nausea, and focal neurological deficits may be additional findings. Physical examination will reveal some degree of meningismus in ~50% of the cases. Gait abnormalities and focal neurologic deficits may be seen in a minority of cases [7].

Hydrocephalus is a late complication of coccidioidal meningitis. Initially, the hydrocephalus may dominate the clinical findings. In persons at risk for coccidioidal infection, hydrocephalus should always be considered a search for the underlying cause, including an evaluation for coccidioidomycosis [9] the lumber puncture for CSF samples requested for the diagnosis and management of coccidioidal meningitis. The CSF parameters are almost always those typical of other chronic meningitides [7].

Case report

A 64 years old diabetic woman with a history of allergic bronchitis at the last three years, she underwent corticosteroid injection during this period of time. Symptoms of meningitis such as fever, nausea, vomiting, and decreasing in mental acuity. She had a history of headaches in several weeks. On admission, her vital signs were as follow: Temperature was 37^{o}C, PR was 80/min., respiratory rate was 15/min., and blood pressure was 110/70 mmHg. In physical examination, she had neck stiffness as well as positive Kernig's signs (positive when the thigh is bent at the hip and knee at 90 degree angles, and subsequent extension in the knee is painful (leading to resistance) and Brudzinski's signs (A positive Brudzinski's sign occurs when flexion of the neck causes involuntary flexion of the knee and hip). Physical examination of chest, heart, abdomen and extremities were normal. She was confused and disoriented to time, place and persons. Cranial nerves examination and deep tendon reflexes were normal and plantar responses was normal. Brain CT scan was normal. CSF parameter reveals lymphocytosis, WBCs were 500, lymphocyte was 90%, PMN was 10%, Glucose was 30 mg/dl, concomitant blood sugar was 155 mg/dl, and a protein was 244 mg/dl. The patient underwent anti-TB treatment due to CSF analysis pattern that improve tuberculosis meningitis, and due to the endemicity of this disease in Iraq. The patient did not respond to anti- TB treatment. Lumber puncture was repeated for direct examination, CSF smear with lactophenol cotton blue, revealed typical picture of *Coccidioidis spp*. (Fig. 1).

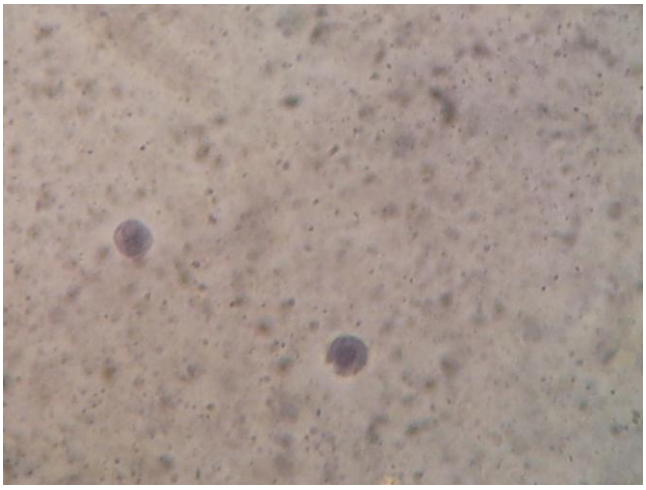

Fig. 1. *Coccidioides* spp. direct smear from CSF, stained with lactophenol cotton blue. Magnification power (400X).

After diagnosis, the patient underwent anti-fungal therapy with IV Amphotericin B 50 mg/day for two weeks. The patient was cured and discharged from the hospital.

Discussion

Coccidioides species one of the most common fungal agents of chronic meningitis in regions endemic with Coccidioidal. Occasionally, even short-term travel to endemic regions results in the acquisition of meningeal disease, so awareness of this complication of coccidioido-mycosis is important even in non-endemic areas.

The prognosis depends on the early recognition and treatment of the disease, so it is important to be familiar with the varied clinical manifestations, risk factors associated with meningeal involvement, diagnostic challenges, and therapeutic modalities [10].

The most predisposing factors lead to develop coccidioidal meningitis is immunosuppressive drugs. History of exposure to *C. immitis*, a wide age range, and, in about one third, underlying conditions are noteworthy. Dissemination to the meninges usually occurs within the first few months although diagnosis is frequently delayed. Presenting symptoms and signs of coccidioidal meningitis are varied but signs of chronic meningitis or suggestion of hydrocephalus are prominent. Evidence of acute infection is unusual even with widespread disease. Diagnosis is usually made by demonstration of coccidioidal CF antibodies in the CSF although they are not found in all patients [11].

The first identified *Coccidioides* species was *Coccidioidesimmitis.* Recently, Taylor *et al*. [12] identified a second species, *Coccidioides-posadasii.* Meningitis is the most squeal form of dissemination and is found in nearly one-half of individuals with disseminated disease. Prior to the advent of anti-fungal treatment, death within a few months was nearly universal.

There are rare reports of survival for 2 years [13]. Most cases of dissemination, including cases of meningitis, occur within weeks to months after primary infection. Rare instances of meningitis presenting years after the original diagnosis of primary or other disseminated disease have been reported [14]. In this study CSF picture and the typical yeasty form of *Coccidioidis spp.* improved the infection with this causative agent.

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